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PCT

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(21) 国際出願番号 PCT/JP96/00374 (22) 国際出願日 1996年2月20日(20.02.96) (30) 優先権データ 特願平7/54977 1995年2月20日(20.02.95) JP 特願平7/207508 1995年7月21日(21.07.95) JP (71) 出願人 (米国を除くすべての指定国について) 雪印乳業株式会社 (SNOW BRAND MILK PRODUCTS CO., LTD.)[JP/JP] 〒065 北海道札幌市東区苗穂町6丁目1番1号 Hokkaido, (JP) (72) 発明者: および (75) 発明者/出願人 (米国についてのみ) 後藤雅昭(GOTO, Masaaki)[JP/JP] 〒329-05 栃木県下都賀郡石橋町下古山456-1 Tochigi, (JP) 津田英資(TSUDA, Eisuke)[JP/JP] 〒329-05 栃木県下都賀郡石橋町石橋622 マロニエハイツ201 Tochigi, (JP) 望月伸一(MOCHIZUKI, Shin'ichi)[JP/JP] 〒329-04 栃木県河内郡南河内町緑5-22-6 Tochigi, (JP) 矢野和樹(YANO, Kazuki)[JP/JP] 〒329-05 栃木県下都賀郡石橋町石橋578-15 西浦ハイツ3-1 Tochigi, (JP) 小林文枝(KOBAYASHI, Fumie)[JP/JP]		島 伸行(SHIMA, Nobuyuki)[JP/JP] 〒329-04 栃木県河内郡南河内町緑4-17-5 Tochigi, (JP) 保田尚孝(YASUDA, Hisataka)[JP/JP] 〒329-04 栃木県河内郡南河内町緑2-3293-46 Tochigi, (JP) 中川信明(NAKAGAWA, Nobuaki)[JP/JP] 〒329-05 栃木県下都賀郡石橋町石橋578-15 西浦ハイツ2-4 Tochigi, (JP) 森永伴法(MORINAGA, Tomonori)[JP/JP] 〒321-02 栃木県下都賀郡壬生町幸町3-11-12 Tochigi, (JP) 上田正次(UEDA, Masatsugu)[JP/JP] 〒350-11 埼玉県川越市今福1672-1 メゾンむさし野719 Saitama, (JP) 東尾侃二(HIGASHIO, Kanji)[JP/JP] 〒350 埼玉県川越市山田1769-10 Saitama, (JP) (74) 代理人 弁理士 藤野清也, 外(FUJINO, Seiya et al.) 〒160 東京都新宿区四谷1丁目2番1号 三浜ビル8階 Tokyo, (JP) (81) 指定国 AU, CA, CN, FI, HU, JP, KR, MX, NO, NZ, RU, US, 欧州特許 (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). 添付公開書類 国際調査報告書
(54) Title : NOVEL PROTEIN AND METHODS FOR THE PRODUCTION OF THE SAME (54) 発明の名称 新規蛋白質及びその製造方法 (57) Abstract A novel protein having the activity of suppressing the differentiation and/or maturation of osteoclasts and methods of the production of the same. This protein is produced from human fetal pulmonary fibroblasts and has a molecular weight of about 60 KD under reductive conditions or about 120 KD under nonreductive conditions. It can be isolated and purified from the culture medium of the above-mentioned cells. Alternatively, it can be produced by genetic engineering techniques. The invention also provides a cDNA for the genetic engineering production of the protein, an antibody showing an affinity specifically for the protein, and a method for assaying the protein with the use of this antibody.		

(57) 要約

破骨細胞の分化及び／又は成熟抑制活性のある蛋白質及びその製造法。

この蛋白質は、ヒト胎児肺線維芽細胞より産生され、還元条件下約60KD、非還元条件下約120KD の分子量をもつ。この蛋白質は該細胞の培養液から単離精製することができる。また、遺伝子工学的に製造することができる。

本発明では、遺伝子工学的に製造するためのcDNA、あるいはこの蛋白質と特異的親和性を示す抗体、この抗体を用いる蛋白質の測定方法も含まれる。

情報としての用途のみ

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明 細 書

新規蛋白質及びその製造方法

技 術 分 野

本発明は、破骨細胞の分化及び／又は成熟を抑制する活性を示す新規な蛋白質、即ち破骨細胞形成抑制因子 (Osteoclastogenesis Inhibitory Factor; OCIF) 及びその製造方法に関する。

従 来 の 技 術

人の骨は絶えず吸収と再形成を繰り返しているが、この過程で中心的な働きをしている細胞が、骨形成を担当する骨芽細胞と骨吸収を担当する破骨細胞である。これらの細胞が担当している、骨代謝の異常により発生する疾患の代表として、骨粗鬆症が挙げられる。この疾患は、骨芽細胞による骨形成を、破骨細胞による骨吸収が上回ることにより発生する疾患である。この疾患の発生メカニズムについては未だ完全には解明されていないが、この疾患は骨の疼痛を発生し、骨の脆弱化による骨折の原因となる疾患である。高齢人口の増加に伴い、骨折による寝たきり老人の発生の原因となるこの疾患は社会問題にもなっており、その治療薬の開発が急務となっている。このような骨代謝異常による骨量減少症は骨吸収の抑制、骨形成の促進、或いはこれらのバランスの改善により治療することが期待される。

骨形成は、骨形成を担当する細胞の増殖、分化、活性化を促進すること、或いは骨吸収を担当する細胞の増殖、分化、活性化を抑制することにより促進することが期待される。近年、このような活性を有する生理活性蛋白質 (サイトカイン) への関心が高まり、精力的な研究が行われている。骨芽細胞の増殖或いは分化を促進するサイトカインとして、線維芽細胞増殖因子ファミリー (fibroblast growth factor ; FGF : Rodan S.B. et al., Endocrinology vol. 121, p1917, 1987)、インシュリン様増殖因子-I (insulin like growth factor-I ; IGF-I : Hock J.M.

et al., Endocrinology vol. 122, p254, 1988)、インシュリン様増殖因子-I (IGF-II : McCarthy T. et al., Endocrinology vol.124, p301, 1989)、アクチビンA (Activin A ; Centrella M. et al., Mol. Cell. Biol. vol. 11, p250, 1991)、トランスフォーミング増殖因子- β (transforming growth factor- β ; Noda M., The Bone, vol. 2, p29, 1988)、バスキュロトロピン (Vasculotropin ; Varonique M. et al., Biochem. Biophys. Res. Commun. vol.199, p380, 1994)、及び異所骨形成因子ファミリー (bone morphogenetic protein ; BMP : BMP-2 ; Yamaguchi, A et al., J. Cell Biol. vol. 113, p682, 1991, OP-1 ; Sampath T. K. et al., J. Biol. Chem. vol. 267, p20532, 1992、Knutsen R. et al., Biochem. Biophys. Res. Commun. vol.194, p1352, 1993) 等のサイトカインが報告されている。

一方、破骨細胞形成、即ち破骨細胞の分化及び／又は成熟を抑制するサイトカインとしては、トランスフォーミング増殖因子- β (transforming growth factor- β ; Chenu C. et al., Proc. Natl. Acad. Sci. USA, vol.85, p5683, 1988) やインターロイキン-4 (interleukin-4 ; Kasano K. et al., Bone-Miner., vol. 21, p179, 1993) 等が報告されている。又、破骨細胞による骨吸収を抑制するサイトカインとしては、カルシトニン (calcitonin ; Bone-Miner., vol.17, p347, 1992)、マクロファージコロニー刺激因子 (macrophage colony-stimulating factor ; Hattersley G. et al. J. Cell. Physiol. vol.137, p199, 1988)、インターロイキン-4 (Watanabe, K. et al., Biochem. Biophys. Res. Commun. vol. 172, p1035, 1990)、及びインターフェロン- γ (interferon- γ ; Gowen M. et al., J. Bone Miner. Res., vol. 1, p469, 1986) 等が報告されている。

これらのサイトカインは、その骨形成の促進や骨吸収の抑制作用による骨量減少症の改善剤となることが期待され、インシュリン様増殖因子-I や異所骨形成因子ファミリーのサイトカイン等、上記のサイトカインの一部については骨代謝改善剤として臨床試験が実施されている。又、カルシトニンは、骨粗鬆症の治療薬、疼痛軽減薬として既に市販されている。

現在、骨に関わる疾患の治療及び治療期間の短縮を図る医薬品として、臨床で

は活性型ビタミンD₃、カルシトニン及びその誘導体、エストラジオール等のホルモン製剤、イブリフラボン、ビタミンK₂（メナテトレノン）又はカルシウム製剤等が使用されている。しかし、これらの薬剤を用いた治療法はその効果並びに治療結果において必ずしも満足できるものではなく、これらに代わる新しい治療薬の開発が望まれていた。前述したように、骨代謝は骨形成と骨吸収のバランスによって調節されており、破骨細胞の分化・成熟を抑制するサイトカインは、骨粗鬆症等の骨量減少症の治療薬となることが期待される。

発明の開示

本発明はこのような観点からなされたものであって、新規な破骨細胞形成抑制因子（OCIF）及びその効率的な製造方法を提供することを課題とする。

本発明者らは、このような現状に鑑み鋭意探索の結果、ヒト胎児肺線維芽細胞IMR-90（ATCC寄託-受託番号CCL186）の培養液に破骨細胞形成抑制活性、即ち破骨細胞の分化・成熟を抑制する活性を有する蛋白質OCIFを見出すに至った。

又、細胞培養の担体としてアルミナセラミック片を使用すると本発明の破骨細胞形成抑制因子OCIFを培地中に高濃度に蓄積せしめ、効率よく精製できることを見出した。

さらに、本発明者らは、前記培養液をイオン交換カラム、アフィニティークラム及び逆相カラムで順次処理して吸着及び溶出をくり返すことによって前記蛋白質OCIFを効率よく精製する方法を確立した。

次に本発明者らは、得られた天然型OCIF蛋白質のアミノ酸配列の情報に基づき、この蛋白質をコードするcDNAのクローニングに成功した。さらに本発明者らは、このcDNAを用いて遺伝子工学的手法により破骨細胞の分化及び、又は成熟抑制活性のある蛋白質を生産する方法を確立するに至った。

本発明は、ヒト胎児肺線維芽細胞に由来し、還元条件下SDS-PAGEにおける分子量が約60kD、非還元条件下SDS-PAGEにおける分子量が約60kD及び約120kDであり、陽イオン交換体及びヘパリンカラムに親和性を有し、70℃、

10分間又は56℃、30分間の加熱処理により破骨細胞の分化・成熟を抑制する活性が低下し、90℃、10分間の加熱処理により破骨細胞の分化・成熟抑制活性が失われることを特徴とする蛋白質に関する。本発明の蛋白質OCIFのアミノ酸配列は、既知の破骨細胞形成抑制因子とは明確に相違する。

また、本発明は、ヒト線維芽細胞を培養し、培養液をヘパリンカラム処理し、吸着画分を溶出し、この画分を陽イオン交換カラムにかけ吸着・溶出し、さらにアフィニティークラム、逆相カラムによって精製して前記蛋白質を採取する、蛋白質OCIFの製造方法に関する。本発明におけるカラム処理は、単に培養液等をヘパリンセファロースカラム等に流下させるものばかりではなく、バッチ法で培養液をヘパリンセファロース等と混合し、カラム処理した場合と同等の効果を奏するものも包含する。本発明で使用されるアフィニティークラムは、ヘパリンカラム及びブルーカラムが挙げられる。ブルーカラムは、特に好ましくはシバクロンブルーカラムが挙げられる。このシバクロンブルーカラムの充填剤としては、親水性合成高分子を担体とし色素シバクロンブルーF3GAを結合させたものが例示され、このカラムは通常ブルーカラムと呼ばれる。

さらに、本発明は、アルミナセラミック片を担体として使用して細胞培養を行なって効率よく前記蛋白質を製造する方法に関する。

本発明の蛋白質OCIFは、ヒト線維芽細胞の培養液から効率良く且つ高収率で単離精製することができる。この原料からの本発明蛋白質OCIFの単離、精製は、生物試料からの蛋白性物質の精製に汎用される通常の方法を用いて、目的とする蛋白質OCIFの物理的、化学的性質を利用した各種の精製操作に従い実施することができる。この濃縮手段として限外濾過、凍結乾燥、及び塩析等の通常の生化学的処理手段が挙げられる。又、精製手段としては、イオン交換クロマトグラフィー、アフィニティークロマトグラフィー、ゲル濾過クロマトグラフィー、疎水クロマトグラフィー、逆相クロマトグラフィー、調製用電気泳動等を用いた通常の蛋白性物質の精製に利用される各種の手法を組み合わせて用いることができる。特に好ましくは、原料として用いるヒト線維芽細胞としてヒト胎児肺線維芽細胞IMR-90(ATCC-CCL 186)を用いることが望ましい。そして原料とな

るヒト胎児肺線維芽細胞 I M R - 90 の培養は、ヒト胎児肺線維芽細胞 I M R - 90 をアルミナセラミック片に付着させ、5 % ウシ新生児血清を添加した D M E M 培地を培養液として用い、ローラーボトル中で一週間から10日程度静置培養することにより得たものを使用するとよい。又、精製処理を実施する際に界面活性剤として0.1 % C H A P S (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) を添加して精製を行うのが望ましい。

本発明の蛋白質 O C I F は、先ず培養液をヘパリンカラム (ヘパリン-セフローズ C L - 6 B、ファルマシア社) にかけて、2M NaCl を含む10mM Tris-HCl 緩衝液、pH7.5 で溶出させ、ヘパリン吸着性の O C I F 画分を得、この画分を Q - 陰イオン交換カラム (HiLoad-Q/FF、ファルマシア社) にかけて、その非吸着画分を集めることにより、ヘパリン吸着性で塩基性の O C I F 画分として得ることができる。得られた O C I F 活性画分は S - 陽イオン交換カラム (HiLoad-S/HP、ファルマシア社)、ヘパリンカラム (ヘパリン-5 P W、トーソー社)、シバクロンブルーカラム (ブルー-5PW、トーソー社)、逆相カラム (BU-300 C4、パーキンエルマー社) にかけることにより単離・精製することができ、この物質は前述した性質によって特定される。

さらに、本発明は、このようにして得られた天然型 O C I F 蛋白質のアミノ酸配列に基づいてこの蛋白質をコードする c D N A をクローニングし、この c D N A を用いて遺伝子工学的手法で破骨細胞の分化及び／又は成熟抑制活性のある蛋白質 O C I F を得る方法に関する。

即ち、本発明の方法に従って精製した O C I F 蛋白質をエンドプロテアーゼ (例えばリシルエンドペプチダーゼ) で処理後、生ずるペプチドのアミノ酸配列を決定し、得られた内部アミノ酸配列をコードし得るオリゴヌクレオチドの混合物を作製する。

次に、作製したオリゴヌクレオチド混合物をプライマーとし、P C R 法 (好ましくは R T - P C R 法) を利用して O C I F c D N A 断片を取得する。この O C I F c D N A 断片をプローブとして、c D N A ライブラリーより O C I F の全長 c D N A をクローニングする。得られた O C I F c D N A を発現ベクターに挿入して O C I F 発現プラス

ミドを作製し、これを各種の細胞又は菌株に導入して発現させることにより、組み換え型OCIFを製造することができる。

本発明はまた、上述の活性を有する本発明OCIF蛋白質の類縁体（バリエーション）である新規蛋白質 OCIF2, OCIF3, OCIF4, OCIF5 に関する。

これらの類縁体は、IMR-90細胞のポリ(A)・RNAを用いて作成したcDNAライブラリーをOCIFcDNA断片をプローブとしてハイブリダイズすることによって得られる。これらのOCIF類縁体のcDNAを発現ベクターに挿入し、そのOCIF類縁体発現ベクターを通常の宿主で発現し、常法で精製することにより、目的とする類縁体蛋白質を得ることができる。

又、本発明はOCIF変異体に関する。

これらの変異体はOCIFの二量体形成に関与する可能性のあるCys 残基をSer 残基に置換したもの、又は天然型OCIFに欠失変異を導入したものである。PCR法或いは制限酵素による切断により、OCIFcDNAに置換或いは欠失変異を導入する。このcDNAを適当な発現プロモーターを有したベクターに挿入し、哺乳動物細胞等の真核細胞にトランスフェクトし、この細胞を培養してその培養液から常法により精製することにより、目的とするOCIF変異体を得られる。

又、本発明は抗OCIFポリクローナル抗体、及びそれを用いたOCIFの測定方法に関する。

抗OCIFポリクローナル抗体は、OCIFを免疫原として常法により作製される。この時用いる抗原（免疫原）としては、IMR-90培養液より得られる天然型OCIF、及びOCIFcDNAを用いて微生物や真核細胞を宿主として生産された遺伝子組み換え型OCIF、あるいはOCIFのアミノ酸配列に基づいて設計した合成ペプチドや、OCIFの加水分解部分ペプチドを用いることができる。これらの抗原を用いて、また必要ならば免疫アジュバントを併用して、適当な哺乳動物を免疫し、その血清から常法により精製することにより、抗OCIFポリクローナル抗体を得ることができる。得られた抗OCIFポリクローナル抗体をアイソトープや酵素で標識することにより、ラジオイムノアッセイ(RIA) やエンザイムイムノアッセイ(EIA) の測定系に使用することができる。この測定系を用い

ることにより、血液や腹水などの生体試料や細胞培養液などのOCIF濃度を容易に測定することができる。

又、本発明は抗OCIFモノクローナル抗体、及びそれを用いたOCIFの測定方法に関する。

抗OCIFモノクローナル抗体は、OCIFを免疫原として、常法により作成される。抗原としては、IMR-90培養液より得られる天然型OCIF、及びOCIFcDNAを用いて微生物や真核細胞を宿主として生産された遺伝子組み換え型OCIF、或いはOCIFのアミノ酸配列に基づいて設計した合成ペプチドや、OCIFの加水分解部分ペプチドでもよい。これらの抗原を用いて哺乳動物を免疫するか、或いはインビトロ法により免疫した細胞を、哺乳動物の骨髄腫細胞（ミエローマ）などと融合させハイブリドーマを作製し、このハイブリドーマよりOCIFを認識する抗体を産生するクローンを選択し、このクローンを培養することにより目的とする抗体が得られる。ハイブリドーマの作製にあたっては、哺乳動物を使用する場合、マウスやラットなどの小動物を使用した例が一般的である。免疫は、OCIFを生理食塩水などにより適当な濃度に希釈し、この溶液を静脈内や腹腔内に投与し、これに必要に応じて免疫アジュバントを併用投与し、動物に2-20日毎に2-5回投与する。このようにして免疫された動物を、解剖し、脾臓を摘出し脾細胞を免疫細胞として使用する。

免疫細胞と細胞融合させるマウス由来のミエローマとしては、例えばP3/x63-Ag8, p3-U1, NS-1, MPC-11, SP-2/0, F0, P3x63Ag8, 653, S194などが例示できる。また、ラット由来の細胞としてはR-210などの細胞株を例示できる。ヒト型の抗体を生産する場合にはヒトBリンパ球をインビトロ法により免疫し、ヒトミエローマ細胞やEBウイルスにより形質転換した細胞株を親株として使用することによりヒト型の抗体を生産するハイブリドーマを得ることができる。

免疫細胞とミエローマ細胞株の融合は公知の方法、例えばKoehlerとMilsteinらの方法（Koehler, G. et al. Nature vol. 256, 495-497, 1975）、或いは電気パルス法などが挙げられる。免疫細胞とミエローマ細胞株は、細胞培養に用いられている培地（FBS不含）に、通常行われている細胞数の比に混合し、ポリエ

チレングリコールを添加して融合処理を行い、HAT選択培地で培養を行い融合細胞を選択することができる。

抗OCIF抗体生産株を選別するには、ELISA法、プラーク法、オクタロニー法、凝集法など、通常の抗体検出に使用されている方法を用いて選択することができる。このようにして選別されたハイブリドーマは、通常の培養方法により継代培養可能であり、必要に応じて凍結保存できる。ハイブリドーマを常法により培養するか、または哺乳動物の腹腔内に移植することにより、抗体を生産することができる。抗体は塩析、ゲル濾過やアフィニティークロマトグラフィーなどの通常の方法により精製できる。

得られた抗体はOCIFに特異的に反応し、OCIFの測定や精製に使用できる。OCIFの測定に使用する場合は、抗体をアイソトープや酵素によりラベルすることにより、ラジオイムノアッセイ(RIA)やエンザイムイムノアッセイ(EIA)の測定系に使用することができる。特に本発明により得られる抗体は、その抗原認識部位がそれぞれ異なっているので、サンドイッチイムノアッセイに使用することができるという特徴を有する。この測定系を用いることにより、血液や腹水などの生体試料や細胞培養液などのOCIF濃度を容易に測定することができる。

OCIF活性は、久米川正好らの方法(蛋白質・核酸・酵素, Vol.34, p999 (1989))及びTakahashi N. et al.の方法(Endocrinology, Vol.122, p1373 (1988))に従い測定することができる。即ち、生後約17日のマウス骨髄細胞を標的細胞として用い、活性型ビタミンD₃(Calcitriol)存在下での破骨細胞の形成抑制を、酒石酸耐性酸性ホスファターゼ活性の誘導の抑制で試験することができる。

本発明の蛋白質である破骨細胞形成抑制因子OCIFは、骨粗鬆症等の骨量減少症、リウマチ又は変形性関節症等の骨代謝異常疾患、或いは多発性骨髄腫等の骨代謝異常疾患の治療及び改善を目的とした医薬組成物として、或いはこのような疾患の免疫学的診断を確立するための抗原として有用である。本発明の蛋白質は、製剤化して経口或いは非経口的に投与することができる。即ち、本発明の蛋

白質を含む製剤は、破骨細胞形成抑制因子 O C I F を有効成分として含む医薬組成物としてヒト及び動物に対して安全に投与されるものである。

医薬組成物の形態としては、注射用組成物、点滴用組成物、坐剤、経鼻剤、舌下剤、経皮吸収剤等が挙げられる。注射用組成物の場合は、本発明の破骨細胞形成抑制因子の薬理学的有効量及び製薬学的に許容しうる担体の混合物であり、その中にはアミノ酸、糖類、セルロース誘導体、及びその他の有機／無機化合物等の一般的に注射用組成物に添加される賦形剤／賦活剤を用いることもできる。又、本発明の破骨細胞形成抑制因子 O C I F とこれらの賦形剤／賦活剤を用い注射剤を調製する場合は、必要に応じて pH 調整剤、緩衝剤、安定化剤、可溶化剤等を添加して常法によって各種注射剤とすることができる。

図面の簡単な説明

第 1 図は、HiLoad-Q/FF 非吸着画分粗精製製品（試料 3）を HiLoad-S/HP カラムにかけた時の溶出プロファイルを示す。

第 2 図は、ヘパリン-5PW 粗精製製品（試料 5）をブルー-5PW カラムにかけた時の溶出プロファイルを示す。

第 3 図は、ブルー-5PW 溶出フラクション 49～50 を逆相カラムにかけた時の溶出プロファイルを示す。

第 4 図は、最終精製品の還元条件下と非還元条件下における SDS-PAGE の結果を示す。

符号の説明

レーン 1、4；分子量マーカー

レーン 2、5；ピーク 6

レーン 3、6；ピーク 7

第 5 図は、還元ピリジルエチル化後、リシルエンドプロテアーゼ処理したピーク 7 を逆相カラムにかけた時の溶出プロファイルを示す。

第 6 図は、天然(n) 及び組み換え型(r) O C I F の、非還元条件下における SDS-PAGE の結果を示す。又、(E) は 293/E B N A 細胞で生産したものを、

(C) はCHO細胞で生産したものをそれぞれ示す。

符号の説明

レーン1 ; 分子量マーカー

レーン2 ; モノマー型nOCIF

レーン3 ; ダイマー型nOCIF

レーン4 ; モノマー型rOCIF (E)

レーン5 ; ダイマー型rOCIF (E)

レーン6 ; モノマー型rOCIF (C)

レーン7 ; ダイマー型rOCIF (C)

第7図は、天然型(n)及び組み換え型(r)OCIFの、還元条件下におけるSDS-PAGEの結果を示す。又、(E)は293/EBNA細胞で生産したものを、(C)CHO細胞で生産したものをそれぞれ示す。

符号の説明

レーン8 ; 分子量マーカー

レーン9 ; モノマー型nOCIF

レーン10 ; ダイマー型nOCIF

レーン11 ; モノマー型rOCIF (E)

レーン12 ; ダイマー型rOCIF (E)

レーン13 ; モノマー型rOCIF (C)

レーン14 ; ダイマー型rOCIF (C)

第8図は、N-結合型糖鎖を除去した天然型(n)及び組み換え型(r)OCIFの、還元条件下におけるSDS-PAGEの結果を示す。又、(E)は293/EBNA細胞で生産したものを、(C)はCHO細胞で生産したものをそれぞれ示す。

符号の説明

レーン15 ; 分子量マーカー

レーン16 ; モノマー型nOCIF

レーン17 ; ダイマー型nOCIF

レーン18 ; モノマー型rOCIF (E)

レーン19 ; ダイマー型 r O C I F (E)

レーン20 ; モノマー型 r O C I F (C)

レーン21 ; ダイマー型 r O C I F (C)

第9図は、O C I F と O C I F 2 の、アミノ酸配列の比較を示す。

第10図は、O C I F と O C I F 3 の、アミノ酸配列の比較を示す。

第11図は、O C I F と O C I F 4 の、アミノ酸配列の比較を示す。

第12図は、O C I F と O C I F 5 の、アミノ酸配列の比較を示す。

第13図は、抗 O C I F ポリクローナル抗体を用いた時の、O C I F の検量線を示す。

第14図は、抗 O C I F モノクローナル抗体を用いた時の、O C I F の検量線を示す。

第15図は、O C I F の骨粗鬆症に対する治療効果を示す。

発明を実施するための最良の形態

以下に実施例を挙げて本発明をさらに詳しく説明する。しかしこれらは単に例示するのみであり、本発明はこれらにより限定されるものではない。

〔実施例1〕

ヒト線維芽細胞 I M R - 90 培養液の調製

ヒト胎児肺線維芽細胞 I M R - 90 (ATCC-CCL186) は、ローラーボトル (490cm²、110 × 171mm、コーニング社) 中で 80g のアルミナセラミック片 (アルミナ 99.5%、東芝セラミック社) に付着させ培養した。培養には 60 個のローラーボトルを使用し、ローラーボトル 1 個当たり 5% 子牛血清を添加した 10mM H E P E S 緩衝液添加 D M E M 培地 (ギブコ B R L 社) 500ml を用い、37℃、5%CO₂ 存在下で 7~10 日間静置培養した。培養後培養液を回収し、新たな培地を添加することにより 1 回の培養で 301 の I M R - 90 培養液を得た。得られた培養液を試料 1 とした。

〔実施例2〕

破骨細胞形成抑制活性の測定法

本発明の蛋白性破骨細胞形成抑制因子の活性測定は久米川正好らの方法（蛋白質・核酸・酵素 Vol.34 p999(1989)）及びTakahashi N. et al. の方法（Endocrinology vol.122 p1373 (1988)）に従い測定した。即ち、生後約17日のマウスより分離した骨髓細胞を用い、活性型ビタミンD₃存在下での破骨細胞形成を酒石酸耐性酸性ホスファターゼ活性の誘導を指標として試験し、その抑制活性を測定することによって行った。即ち、96ウェルマイクロプレートに 2×10^{-8} M活性型ビタミンD₃及び10%牛胎児血清を含む α -MEM培地（ギブコBRL社）で希釈したサンプル100 μ lを入れ、生後約17日のマウスから得た骨髓細胞 3×10^5 個を100 μ lの10%牛胎児血清を含む α -MEM培地に懸濁させて播種し、5%CO₂、37℃、湿度100%にて一週間培養した。培養3日目と5日目に、培養液160 μ lを廃棄し、 1×10^{-8} M活性型ビタミンD₃及び10%牛胎児血清を含む α -MEM培地で希釈したサンプル160 μ lを添加した。培養7日後にリン酸塩緩衝生理食塩水で洗浄した後エタノール/アセトン（1：1）溶液で細胞を室温にて1分間固定し、破骨細胞形成を酸性ホスファターゼ活性測定キット（Acid Phosphatase, Leucocyte、カタログNo.387-A、シグマ社）を用いた染色で検出した。酒石酸存在下での酸性ホスファターゼ活性陽性細胞の減少をOCIF活性とした。

〔実施例3〕

OCIFの精製

i) ヘパリン・セファロースCL-6Bによる精製

約90lのIMR-90培養液（試料1）を、0.22 μ mのフィルター（親水性ミリディスク、2,000cm²、ミリポア社）で濾過した後、3回に分けて0.3M NaClを含む10mM Tris-HCl 緩衝液（以下、Tris-HClという）、pH7.5で平衡化させたヘパリン・セファロースCL-6Bカラム（5×4.1cm、ゲル容量80ml）にかけた。流速500ml/hrにて、10mM Tris-HCl、pH7.5で洗浄した後、2M NaClを含む10mM Tris-HCl、pH7.5で溶出を行い、ヘパリン・セファロースCL-6B吸着画分900mlを得、得られた画分を試料2とした。

ii) Hi Load-Q/FFによる精製

ヘパリン・セファロース吸着画分（試料2）を10mM Tris-HCl、pH7.5に対し

て透析した後、0.1 %になるようにCHAPSを加え4℃で一晩放置したものを、2回に分けて0.1 % CHAPSを含む50mM Tris-HCl、pH7.5で平衡化した陰イオン交換カラム(HiLoad-Q/FF、2.6 × 10cm、ファルマシア社)にかけ、非吸着画分1000mlを得た。得られた画分を試料3とした。

iii) HiLoad-S/HPによる精製

HiLoad-Q非吸着画分(試料3)を、0.1 % CHAPSを含む50mM Tris-HCl、pH7.5で平衡化した陽イオン交換カラム(HiLoad-S/HP、2.6 × 10cm、ファルマシア社)にかけた。0.1 % CHAPSを含む50mM Tris-HCl、pH7.5で洗浄した後、100分間でNaClを1Mにする直線勾配、流速8ml/分にて溶出を行い、12ml/フラクションにて分取を行った。フラクション1~40を10フラクションづつ4つの画分にまとめ、それぞれ100μlを用いてOCIF活性を測定した。OCIF活性はフラクション11~30に認められた(図1: 図中、++は破骨細胞形成が80%以上抑制される活性を、+は破骨細胞形成が30~80%抑制される活性を、-は活性が検出されないことをそれぞれ示す)。より比活性の高いフラクション21~30を試料4とした。

iv) アフィニティーカラム(ヘパリン-5PW)による精製

120mlの試料4を240mlの0.1 % CHAPSを含む50mM Tris-HCl、pH7.5で希釈した後、0.1 % CHAPSを含む50mM Tris-HCl、pH7.5で平衡化したアフィニティーカラム(ヘパリン-5PW、0.8 × 7.5 cm、トーソー社)にかけた。0.1 % CHAPSを含む50mM Tris-HCl、pH7.5で洗浄した後、60分間でNaClを2Mにする直線勾配、流速0.5ml/分にて溶出を行い、0.5ml/フラクションにて分取を行った。各フラクション50μlを用いてOCIF活性を測定し、約0.7~1.3M NaClで溶出されるOCIF活性画分10mlを得、試料5とした。

v) アフィニティーカラム(ブルー-5PW)による精製

10mlの試料5を190mlの0.1 % CHAPSを含む50mM Tris-HCl、pH7.5で希釈した後、0.1 % CHAPSを含む50mM Tris-HCl、pH7.5で平衡化したアフィニティーカラム(ブルー-5PW、0.5 × 5.0cm、トーソー社)にかけた。0.1 % CHAPSを含む50mM Tris-HCl、pH7.5で洗浄した後、60分間でNaClを2Mにする直線

勾配、流速0.5ml/分にて溶出を行い、0.5ml/フラクションにて分取を行った。各フラクション25 μ lを用いてOCIF活性を測定し、約1.0～1.6M NaClで溶出されるOCIF活性フラクション49～70を得た（図2 図中、++は破骨細胞形成が80%以上抑制される活性を、+は破骨細胞形成が30～80%抑制される活性を示す）。

vi) 逆相カラムによる精製

得られたフラクション49～50mlに、10 μ lの25%TFA（トリフルオロ酢酸）を加えた後、0.1%TFAを含む25%アセトニトリルで平衡化した逆相カラム（BU-300、C4、2.1 \times 220mm、パーキンエルマー社）にかけ、60分間でアセトニトリルを55%にする直線勾配、流速0.2ml/分にて溶出を行い、各ピークを分取した（図3）。各ピークフラクションの100 μ lを用いてOCIF活性を測定し、ピーク6及びピーク7に濃度依存的に活性を検出した。結果を表1に示す。

第1表 逆相カラムから溶出されたOCIF活性

希釈率	1/40	120	1/360	1/1080
ピーク6	++	++	+	—
ピーク7	++	+	—	—

（表中、++は破骨細胞形成が80%以上抑制される活性を、+は破骨細胞形成が30～80%抑制される活性を、—は活性が検出されないことを示す。）

〔実施例4〕

OCIFの分子量測定

OCIF活性の認められたピーク6及びピーク7各40 μ lを用い、還元条件下と非還元条件下でSDS-ポリアクリルアミドゲル電気泳動を行った。即ち、各ピークフラクション20 μ lづつを2本のチューブに分取し減圧濃縮した後、1mM EDTA、2.5%SDS、及び0.01%ブロモフェノールブルーを含む10mM Tris-HCl, pH8 1.5 μ lで溶解し、それぞれを非還元条件下及び還元条件下（5% 2-メルカプトエタノール存在下）で37℃で一晩放置後、それぞれの1 μ lをSDS

ーポリアクリルアミドゲル電気泳動に負荷した。電気泳動は10-15%アクリルアミドのグラジエントゲル(ファルマシア社)を使用し、電気泳動装置Phast System(ファルマシア社)を用いて行った。分子量マーカーとして、ホスホリラーゼb(94kD)、ウシ血清アルブミン(67kD)、オボアルブミン(43kD)、カルボニックアンヒドラーゼ(30kD)、トリプシンインヒビター(20.0kD)、 α -ラクトアルブミン(14.4kD)を用いた。電気泳動終了後、Phast Gel Silver Stain Kit(ファルマシア社)を用いて銀染色を行った。結果を図4に示す。

その結果、ピーク6については還元条件下、非還元条件下で約60kDの蛋白質のバンドが検出された。又、ピーク7については、還元条件下で約60kD、非還元条件下で約120kDaの蛋白質のバンドが検出された。従って、ピーク7はピーク6の蛋白質のホモダイマーであると考えられる。

[実施例5]

OCIFの熱安定性試験

ブルー5PWフラクション51~52を混合したサンプルから20 μ lずつを取り、10mMリン酸塩緩衝生理食塩水、pH7.2 30 μ lを加えた後、70℃及び90℃にて10分間、又は56℃にて30分間熱処理を行った。このサンプルを用い、実施例2記載の方法に従いOCIF活性を測定した。結果を表2に示す。

第2表 OCIFの熱安定性

希 釈 率	1/300	1/900	1/2700
未処理	++	+	-
70℃10分	+	-	-
56℃30分	+	-	-
90℃10分	-	-	-

(表中、++は破骨細胞形成が80%以上抑制される活性を、+は破骨細胞形成が30~80%抑制される活性を、-は活性が検出されないことを示す。)

BAD ORIGINAL

〔実施例 6〕

内部アミノ酸配列の決定

ブルー-5 PWフラクション51~70について、2フラクションづつを混合して1 mlとし、それぞれの試料に10 μ l の25% TFAを加えた後、1 mlずつ10回にわけて0.1 % TFAを含む25%アセトニトリルで平衡化した逆相カラム (BU-300、C4、2.1 \times 220mm、パーキンエルマー社) につけ、60分間でアセトニトリルを55%にする直線勾配、流速 0.2 ml/分にて溶出を行い、ピーク6とピーク7を集めた。得られたピーク6とピーク7の一部について、それぞれプロテインシーケンサー (プロサイス、494 型、パーキンエルマー社) を用い、N末端アミノ酸配列分析を行ったが、分析不能でありこれらの蛋白質のN末端はブロックされている可能性が示唆された。そこで、これらの蛋白質の内部アミノ酸配列を解析した。即ち、ピーク6とピーク7のそれぞれを遠心濃縮した後、それぞれに 100 μ g ジチオスレイトール、10mM EDTA、7 M塩酸グアニジン、及び1% CHAPSを含む 0.5M Tris-HCl, pH8.5 50 μ l を加えて室温で4時間放置し還元した後、0.2 μ l の4-ビニルピリジンを加え、室温暗所で一晚放置しビリジルエチル化した。これらのサンプルに1 μ l の25% TFAを加え、0.1 % TFAを含む20%アセトニトリルで平衡化した逆相カラム (BU-300, C4, 2.1 \times 30mm, パーキンエルマー社) につけ、30分間でアセトニトリル濃度を50%にする直線勾配、流速0.3 ml/分で溶出を行い、還元ビリジルエチル化OCIFサンプルを得た。還元ビリジルエチル化したサンプルのそれぞれを遠心濃縮し、8M尿素及び0.1% Tween80を含む0.1M Tris-HCl, pH9 25 μ l で溶解した後、73 μ l の0.1M Tris-HCl, pH9 で希釈し、0.02 μ g のAP1 (リシルエンドプロテアーゼ、和光純薬社) を加え、37 $^{\circ}$ Cで15時間反応させた。反応液に1 μ l の25% TFAを加え、0.1 % TFAで平衡化した逆相カラム (RP-300, C8, 2.1 \times 220mm、パーキンエルマー社) につけ、70分間でアセトニトリル濃度を50%にする直線勾配、流速0.2 ml/分で溶出を行い、ペプチドフラグメントを得た (図5)。得られたペプチドフラグメント (P1~P3) について、プロテインシーケンサーを用いアミノ酸配列分析を行った。結果を配列表 配列番号1~3に示す。

END ORIGINAL

〔実施例 7〕

cDNA配列の決定i) IMR-90細胞からのポリ(A)・RNAの単離

IMR-90細胞のポリ(A)・RNAは、ファストトラックmRNAアイソレーションキット（インヴィトロジェン社）を用い、そのマニュアルに準じて単離した。この方法により 1×10^8 個のIMR-90細胞より約 $10 \mu\text{g}$ のポリ(A)・RNAを取得した。

ii) ミックスプライマーの作製

先に得られたペプチド（配列表 配列番号2及び3）のアミノ酸配列をもとに、次の2種のミックスプライマーを合成した。即ち、ペプチドP2（配列番号2のペプチド）の6番目(Gln)から12番目(Leu)までのアミノ酸配列をコードしうるすべての塩基配列を持つオリゴヌクレオチドの混合物（ミックスプライマー、No.2F）を合成した。又、ペプチドP3（配列番号3のペプチド）の6番目(His)から12番目(Lys)までのアミノ酸配列をコードしうるすべての塩基配列に対する相補的オリゴヌクレオチドの混合物（ミックスプライマー、No.3R）を合成した。用いたミックスプライマーの塩基配列を、表3に示す。

第3表

=No.2F =

5'-CAAGAACAAA CTTTCAATT-3'
 G G G C C GC
 A
 G

=No.3R =

5'-TTTATACATT GTAAAAGAAT G-3'
 C G C G GCTG
 A C
 G T

iii) OCIFcDNA断片のPCRによる増幅

実施例7-i)で得たポリ(A)・RNA、 $1 \mu\text{g}$ を鋳型としてスーパースクリプトII cDNA合成キット（ギブコBRL社）を用いて、同社のプロトコールに従っ

て一本鎖 cDNA を合成し、この cDNA と実施例 7 - ii) で示したプライマーを用いて、PCR を行い、OCIFcDNA 断片を取得した。以下に条件を示す。

10X Ex Taq バッファー (宝酒造社)	5	μl
2.5 mM dNTP	4	μl
cDNA 溶液	1	μl
Ex Taq (宝酒造社)	0.25	μl
蒸留水	29.75	μl
40 μM プライマー No. 2F	5	μl
40 μM プライマー No. 3R	5	μl

上記の溶液を微量遠心チューブ中で混合後、以下の条件で PCR を行った。95℃ で 3 分前処理後、95℃ 30 秒、50℃ 30 秒、70℃ 2 分の 3 段階の反応を 30 回繰り返したのち、70℃ 5 分保温した。反応液の一部をアガロース電気泳動し約 400bp の均一な DNA 断片が得られたことを確認した。

[実施例 8]

PCR により増幅された OCIFcDNA 断片のクローニング及び塩基配列決定

実施例 7 - iii) で得られた OCIFcDNA 断片を、Marchuk, D の方法 (Nucleic Acid Res., Vol. 19, p1154, 1991) によってプラスミド pBluescript II SK⁻ (ストラタジーン社) に DNA ライゲーションキット Ver. 2 (宝酒造社) を用いて挿入し、大腸菌 DH5α (ギブコ BRL 社) の形質転換を行った。得られた形質転換株を増殖させ、約 400bp の OCIFcDNA 断片が挿入されたプラスミドを常法に従い精製した。このプラスミドを pBSOCIF と名付け、このプラスミドに挿入されている OCIFcDNA の塩基配列をタックダイデオキシターミネーターサイクルシーケンシングキット (Taq Dye Deoxy Terminator Cycle Sequencing kit; パーキンエルマー社) を用いて決定した。この OCIFcDNA の大きさは、397 bp であった。この塩基配列から予測される 132 個のアミノ酸からなるアミノ酸配列中に、ミックスプライマーを設計するのに用いた OCIF の内部アミノ酸配列 (配列表配列番号 2 及び 3) をそれぞれ N 末側、C 末側に見出すことができた。又、OCIF の内部アミノ酸配列 (配列番号 1) を、この 132 個のアミノ酸からなるアミノ酸配列中に

見出すことができた。以上の結果より、クローニングした397 bpの cDNA は、OCIFcDNA断片であることが確認された。

〔実施例 9〕

DNAプローブの作製

実施例 8 で作成された397bp のOCIFcDNA断片が挿入されたプラスミドを鋳型にして実施例 7 - iii) の条件でPCRを行なうことにより、このOCIFcDNA断片を増幅した。アガロース電気泳動により397bp のOCIFcDNA断片を分離後、QIAEX ゲルエクストラクションキット（キアゲン社）を用いて精製した。このDNA をメガプライムDNAラベリングキット（アマシャム社）を用いて [α - 32 P]dCTP で標識し、全長のOCIFcDNAをスクリーニングするためのプローブとして用いた。

〔実施例 10〕

cDNAライブラリーの作成

実施例 7 - i) で得られたポリ(A)・RNA、2.5 μ g を鋳型としてグレートレングス cDNA 合成キット（クロンテック社）を用いて同社のプロトコールに従い、oligo(dT)primer を用いて cDNA の合成、EcoRI-SalI-NotI アダプター付加、cDNA サイズフラクショネーションを行いエタノール沈殿の後10 μ l のTEバッファーに溶解した。得られたアダプター付加 cDNA、0.1 μ g をT4DNA リガーゼを用いてあらかじめEcoRI で切断した1 μ g の λ ZAP エクスプレスベクター（ストラタジーン社）に挿入した。このようにして得られた cDNA 組み換えファージDNA 溶液をギガバックゴールドII（ストラタジーン社）を用いてインヴィトロパッケージング反応に供し、 λ ZAP エクスプレス組み換えファージを作成した。

〔実施例 11〕

組み換えファージのスクリーニング

実施例 10 で得られた組み換えファージを37℃で15分間大腸菌 XL1-Blue MRF'（ストラタジーン社）に感染させたのち、50℃に加温した0.7 %の寒天を含むNZY 培地に添加し、NZY 寒天培地プレートに流しこんだ。37℃で一晩培養後、プラークの生じたプレート上にハイボンドN（アマシャム社）を約30秒密着させた。

このフィルターを常法に従いアルカリ変性した後、中和し、2XSSC 溶液に浸したのちUVクロスリンク（ストラタジーン社）によりDNA をフィルターに固定化した。得られたフィルターを100 μ g/mlのサケ精子DNA を含むハイブリダイゼーションバッファー（アマシャム社）に浸漬し65℃で4 時間前処理した後、熱変性した上記DNA プローブ(2X10⁵cpm/ml) を添加した上記バッファーに移し替え65℃で一晩ハイブリダイゼーションを行った。反応後フィルターを2XSSC で2 回、0.1XSSC、0.1% SDS溶液で2回それぞれ65℃で10分間洗浄した。得られたいくつかの陽性クローンを、さらに2 回スクリーニングを行うことにより純化した。それらの中から約1.6kb のインサートを持つものを以下に用いた。この純化したファージを λ OCIFと名付けた。純化した λ OCIFを λ ZAP エクスプレスクローニングキット（ストラタジーン社）のプロトコールに従い、大腸菌XL1-Blue MRF' に感染させたのち、ヘルパーファージExAssist（ストラタジーン社）で多重感染を行い、その培養上清を大腸菌XL0LR（ストラタジーン社）に感染させたのちカナマイシン耐性株を拾うことによりpBKCMV（ストラタジーン社）に上述の1.6kb のインサートが挿入されたプラスミドpBKOCIF をもつ形質転換株を得た。この形質転換株はpBK/01 F10 として、通商産業省工業技術院生命工学工業技術研究所に受託番号FERM BP-5267（平成7 年10月25日にFERM P-14998の原寄託よりブタベスト条約に基づく寄託に移管）として寄託してある。このプラスミドをもつ形質転換株を増殖させ、常法によりプラスミドを精製した。

〔実施例 1 2〕

OCIFの全アミノ酸配列をコードするcDNAの塩基配列の決定

実施例 1 1 で得られたOCIFcDNAの塩基配列をタックダイデオキシターミネーターサイクルシーケンシングキット（パーキンエルマー社）を用いて決定した。用いたプライマーはT3, T7 プライマー（ストラタジーン社）及びOCIFcDNAの塩基配列に基づいて設計された合成プライマーであり、その配列を配列表配列番号16～29に示す。

決定されたOCIFの塩基配列を配列番号6 に、その配列から推定されるアミノ酸配列を配列番号5 にそれぞれ示す。

〔実施例 1 3〕

2 9 3 / E B N A 細胞による組み換え型 O C I F の生産i) OCIFcDNAの発現プラスミドの作製

実施例 1 1 で得られた約 1.6kb の OCIFcDNA が挿入されたプラスミド pBKOCIF を制限酵素 BamHI 及び XhoI で消化し、OCIFcDNA を切り出し、アガロース電気泳動によって分離後、Q I A E X ゲルエクストラクションキット（キアゲン社）を用いて精製した。この OCIFcDNA を、あらかじめ制限酵素 BamHI 及び XhoI で消化しておいた発現プラスミド pCEP4 （インヴィトロジェン社）に、ライゲーションキット Ver.2 （宝酒造社）を用いて挿入し、大腸菌 DH5 α （ギブコ B R L 社）の形質転換を行った。得られた形質転換株を増殖させ、OCIFcDNA が挿入された発現プラスミド pCEPOCIF をキアゲンカラム（キアゲン社）を用いて精製した。O C I F 発現プラスミド pCEPOCIF をエタノールによって沈澱させた後、無菌蒸留水に溶解し以下の操作に用いた。

ii) OCIFcDNA のトランジェントな発現及びその活性の測定

実施例 1 3 - i) で得られた O C I F 発現プラスミド pCEPOCIF を用いて、以下に述べる方法で組み換え O C I F を発現させ、その活性を測定した。8 $\times 10^5$ 個の 293/EBNA 細胞（インヴィトロジェン社）を 6 ウェルプレートの各ウェルに 10% 牛胎児血清（ギブコ B R L 社）を含む I M D M 培地（ギブコ B R L 社）を用いて植え込み、翌日、培地を除いた後、無血清 I M D M 培地で細胞を洗った。トランスフェクション用試薬 リポフェクタミン（ギブコ B R L 社）添付のプロトコールに従い、あらかじめ O P T I - M E M 培地（ギブコ B R L 社）を用いて希釈しておいた pCEPOCIF と リポフェクタミン を混合した後、この混合液を各ウェルの細胞に加えた。用いた pCEPOCIF 及び リポフェクタミン の量はそれぞれ 3 μ g 及び 12 μ l であった。3 8 時間後、培地を除き 1 ml の新しい O P T I - M E M 培地を加え、さらに 3 0 時間後、培地を回収し、これを O C I F 活性測定用サンプルとした。O C I F の活性測定は以下のようにして行った。生後約 1 7 日のマウス骨髄細胞からの活性型ビタミン D₃ 存在下での破骨細胞形成を酒石酸耐性酸性ホスファターゼ活性の誘導で試験し、その抑制活性を測定し、O C I F の活性とした。すなわ

ち、96ウェルマイクロプレートに 2×10^{-8} M 活性型ビタミン D₃ 及び 10% 牛胎児血清を含む α -MEM 培地（ギブコ BRL 社）で希釈したサンプル 100 μ l を入れ、生後約 17 日のマウス骨髄細胞 3×10^5 個を 100 μ l の 10% 牛胎児血清を含む α -MEM 培地に懸濁させて播種し、5% CO₂、37℃、湿度 100% にて一週間培養した。培養 3 日目と 5 日目に、培養液 160 μ l を廃棄し、 1×10^{-8} M 活性型ビタミン D₃ 及び 10% 牛胎児血清を含む α -MEM 培地で希釈したサンプル 160 μ l を添加した。培養 7 日後にリン酸塩緩衝生理食塩水で洗浄した後エタノール／アセトン（1：1）溶液で細胞を室温にて 1 分間固定し、破骨細胞形成を酸性ホスファターゼ活性測定キット（Acid Phosphatase, Leucocyte、カタログ No. 387-A、シグマ社）を用いた染色で検出した。酒石酸存在下での酸性ホスファターゼ活性陽性細胞の減少を OCIF 活性とした。その結果、表 4 に示すように、先に IMR-90 の培養液から得られた天然型 OCIF と同様の活性をこの培養液が有することが確認された。

第 4 表 293/EBNA 細胞で発現させた培養液中の OCIF 活性

希釈率	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
OCIF							
遺伝子導入	++	++	++	++	++	+	-
ベクター導入	-	-	-	-	-	-	-
未処理	-	-	-	-	-	-	-

（表中、++ は破骨細胞形成が 80% 以上抑制される活性を、+ は破骨細胞形成が 30～80% 抑制される活性を、- は活性が検出されないことを示す。）

iii) 293/EBNA 細胞由来組み換え型 OCIF の精製

実施例 13-ii) に記載した 293/EBNA 細胞を大量培養して得た培養液 1.8l に 0.1% になるように CHAPS を加え、0.22 μ m のフィルター（ステリベックス GS、ミリポア社）で濾過した後、10mM Tris-HCl, pH7.5 で平衡化させた 50ml のヘパリン・セファロース CL-6B カラム（2.6×10cm、ファルマシア社）にか

けた。0.1 % CHAPSを含む10mM Tris-HCl, pH7.5 で洗浄した後、100 分間でNaClを2Mにする直線勾配、流速4 ml/分にて溶出を行い、8ml/フラクションにて分取を行った。各フラクション150 μ l を用いて実施例2の方法に従ってOCIF活性を測定し、約0.6~1.2M NaCl で溶出されるOCIF活性画分112mlを得た。

得られたOCIF活性画分112mlを0.1 % CHAPSを含む10mM Tris-HCl, pH7.5 で1000mlに希釈した後、0.1 % CHAPSを含む10mM Tris-HCl, pH7.5 で平衡化させたアフィニティカラム（ヘパリン-5PW, 0.8×7.5 cm、トーソー社）にかけた。0.1 % CHAPSを含む10mM Tris-HCl, pH7.5 で洗浄した後、60分間でNaClを2Mにする直線勾配、流速0.5ml/分にて溶出を行い、0.5ml/フラクションにて分取を行った。

得られたフラクション各4 μ l を用いて実施例4の方法に従って還元及び非還元条件下でSDS-ポリアクリルアミドゲル電気泳動を行った。その結果、フラクション30~32には還元条件下で約60kD、非還元条件下で約60kDと約120kDのOCIFバンドのみが検出されたので、フラクション30~32を集め純化293/EBNA細胞由来組み換え型OCIF (rOCIF(E)) 画分とした。BSAをスタンダードとして用いたローリー法による蛋白定量の結果、535 μ g/mlのrOCIF(E)1.5ml が得られたことが明らかになった。

〔実施例14〕

CHO細胞による組み換え型OCIFの生産

i) OCIFの発現プラスミドの作製

実施例11で得られた約1.6kbのOCIFcDNAが挿入されたプラスミドpBKOCIFを制限酵素SalI及びEcoRVで消化し、約1.4kbのOCIFcDNA断片を切り出し、アガロース電気泳動によって分離後、QIAEX ゲルエクストラクションキット（キアゲン社）を用いて精製した。又、発現ベクターpcDL-SR α 296 (Molecular and Cellular Biology, Vol.8, pp466-472, 1988) を制限酵素PstI及びKpnIで消化し、約3.4kbの発現ベクターDNA断片をアガロース電気泳動によって分離後、QIAEX ゲルエクストラクションキット（キアゲン社）を用いて精製した。D

NAブランディングキット（宝酒造社）を用いて、これらの精製したOCIFcDNA断片と発現ベクターDNA断片の末端を平滑化した。次に、ライゲーションキット Ver.2（宝酒造社）を用いて、平滑化された発現ベクターDNA断片にOCIFcDNA断片を挿入し、大腸菌DH5 α （ギブコBRL社）の形質転換を行い、OCIF発現プラスミドpSR α OCIFをもつ形質転換株を得た。

ii) 発現プラスミドの調製

実施例13-i)で得られたOCIF発現プラスミドpSR α OCIFをもつ形質転換株及びWO92/01053号公報に示されるマウスDHFR遺伝子発現プラスミドpBAdDSVをもつ形質転換株をそれぞれ常法を用いて増殖させ、Maniatisら（Molecular cloning, 2nd edition）の方法に従いアルカリ法及びポリエチレングリコール法で処理し、塩化セシウム密度勾配遠心法により精製した。

iii) CHOdhFr⁻細胞の蛋白質不含培地への馴化

10%牛胎児血清（ギブコBRL社）を含むIMDM培地（ギブコBRL社）で継代されていたCHOdhFr⁻細胞（ATCC-CRL9096）は、無血清培地EX-CELL301（JRHバイオサイエンス社）で馴化後、さらに蛋白質不含培地EX-CELL PF CHO（JRHバイオサイエンス社）で馴化させた。

iv) OCIF発現プラスミド及びDHFR発現プラスミドのCHOdhFr⁻細胞への導入

実施例14-ii)で調製したOCIF発現プラスミドpSR α OCIF及びDHFR発現プラスミドpBAdDSVを用いて実施例14-iii)で調製したCHOdhFr⁻細胞を下記に示すエレクトロポレーション法により形質転換した。pSR α OCIFプラスミド200 μ gとpBAdDSVプラスミド20 μ gを無菌的に10%牛胎児血清（ギブコBRL社）を含むIMDM培地（ギブコBRL社）0.8mlに溶解後、この0.8mlを用いて 2×10^7 個のCHOdhFr⁻細胞を浮遊させた。この細胞浮遊液をキューベツト（バイオラッド社）に入れ、ジーンバルサー（バイオラッド社）を用いて、360V、960 μ Fの条件でエレクトロポレーション法により形質転換を行った。10mlのEX-CELL PF CHO培地の入った浮遊細胞用Tフラスコ（住友ベークライト社）にエレクトロポレーション済の細胞浮遊液を移し、CO₂インキュベーター中で2日間培

養した。EX-CELL PF CHO培地を用いて5000cells/wellの濃度で96ウェルマイクロプレートにまき、約2週間培養した。EX-CELL PF CHO培地を核酸は含まず、この培地では親株のCHOdhFr⁻は増殖できないので、DHFRを発現する細胞株だけが選択されてくる。OCIF発現プラスミドをDHFR発現プラスミドの10倍量用いているので、DHFRを発現する細胞株の大部分はOCIFを発現する。得られたDHFRを発現する細胞株から培養上清中のOCIF活性の高い細胞株を、実施例2で示した測定法によってスクリーニングした。得られたOCIF高生産株につきEX-CELL PF CHO培地を用いて限界希釈法により細胞のクローニングを行い、得られたクローンについて培養上清中のOCIF活性の高い細胞株をスクリーニングし、OCIF高生産クローン5561を得た。

v) 組み換え型OCIFの生産

組み換えOCIF (rOCIF) の生産するため、EX-CELL 301 培地3lに形質転換CHO細胞 (5561) を 1×10^5 cells/ml となるように接種し、スピナーフラスコを用いて37℃で4、5日培養した。細胞の濃度が約 1×10^6 cells/ml になったところで、約2.7lの培地を回収した。約2.7lのEX-CELL 301 培地を加え、培養を繰り返した。3基のスピナーフラスコを用い、約20lの培養液を採取した。

vi) CHO細胞由来組み換え型OCIFの精製

実施例14-(v) で得られた培養液1lに0.1%になるようにCHAPSを加え、0.22μmのフィルター(ステリベックスGS、ミリポア社)で濾過した後、10mM Tris-HCl, pH7.5で平衡化させた50mlのヘパリン・セファロースFFカラム(2.6×10cm、ファルマシア社)にかけた。0.1%CHAPSを含む10mM Tris-HCl, pH7.5で洗浄した後、100分間でNaClを2Mにする直線勾配、流速4ml/分にて溶出を行い、8ml/フラクションにて分取を行った。各フラクション150μlを用いて実施例2の方法に従ってOCIF活性を測定し、約0.6~1.2Mで溶出されるOCIF活性画分112mlを得た。

得られたOCIF活性画分112mlを0.1%CHAPSを含む10mM Tris-HCl, pH7.5で1200mlに希釈した後、0.1%CHAPSを含む10mM Tris-HCl, pH7.5で平衡化させたアフィニティカラム(ブルー-5PW, 0.5×5cm、トーソー社)に

かけた。0.1 % CHAPSを含む 10mM Tris-HCl, pH7.5 で洗浄した後、90分間でNaClを3Mにする直線勾配、流速0.5ml/分にて溶出を行い、0.5ml/フラクションにて分取を行った。

得られたフラクション各4 μ l を用いて実施例4の方法に従って還元及び非還元条件下でSDS-ポリアクリルアミドゲル電気泳動を行った。その結果、フラクション30~38には還元条件下で約60kD、非還元条件下で約60kDと約120kDのOCIFバンドのみが検出されたので、フラクション30~38を集め精製CHO細胞由来組み換え型OCIF〔rOCIF(C)〕画分とした。BSAをスタンダードとしたローリー法による蛋白定量の結果、113 μ g/mlのrOCIF(C) 4.5 mlが得られたことが明らかになった。

〔実施例15〕

組み換え型OCIFのN末端構造解析

3 μ gの精製rOCIF(E)及びrOCIF(C)を、プロスピン(ProSpin; パーキンエルマー社)を用いてポリビニリデンジフルオリド(PVDF)膜に固定し、20%メタノールで洗浄した後、プロテインシーケンサー(プロサイス、492型、パーキンエルマー社)を用いてN末端アミノ酸配列分析を行った。結果を配列表配列番号7に示す。

rOCIF(E)と rOCIF(C)のN末端アミノ酸は、配列表配列番号5に記載したアミノ酸配列の翻訳開始点 Metから22番目の Gluで、Met から Glnまでの21アミノ酸はシグナルペプチドであることが明らかになった。又、IMR-90培養液から精製し得られた天然型OCIFのN末端アミノ酸配列が分析不能であったのは、N末端のGluが培養中又は精製中にピログルタミン酸に変換したためと考えられた。

〔実施例16〕

組み換え型(r)OCIF及び天然型(n)OCIFの生物活性

i) マウス骨髄細胞系での、ビタミンD₃で誘導される破骨細胞形成の抑制

96ウェルマイクロプレートに、 2×10^{-8} M活性型ビタミンD₃及び10%牛胎児血清を含む α -MEM培地(ギブコBRL社)で250ng/mlから連続的に二分の一希釈した精製rOCIF(E)及び nOCIF 100 μ lを入れた。このウェルに生後約17日の

マウス骨髄細胞 3×10^5 個を $100 \mu\text{l}$ の10%牛胎児血清を含む α -MEM培地に懸濁させて播種し、5% CO_2 、 37°C 、湿度 100%にて一週間培養した。培養7日後に、実施例2の方法に従って酸性ホスファターゼ活性測定キット(Acid Phosphatase, Leucocyte、カタログNo.387-A、シグマ社)を用いた染色を行い破骨細胞形成を検出した。酒石酸存在下での酸性ホスファターゼ活性陽性細胞の減少をOCIF活性とした。酸性ホスファターゼ活性陽性細胞の減少率は、染色した細胞の色素を可溶化し、その吸光度を測定することにより算出した。即ち、細胞を固定し染色した各ウェルに0.1N水酸化ナトリウム-ジメチルスルフォキシド混合液(1:1) $100 \mu\text{l}$ を加えよく振盪した。色素を十分に溶解させた後、マイクロプレートリーダー(イムノリーダーNJ-2000、インターメッド社)を用い、測定波長 590nm 、対照波長 490nm にて吸光度を測定した。又、吸光度を測定する際のブランクウェルとして、ビタミン D_3 未添加のウェルを用いた。結果は、OCIF未添加のウェルでの吸光度値を100とした百分率値で表し、表5に示す。

第5表 マウス骨髄細胞系でのOCIFによる
破骨細胞形成抑制(ビタミン D_3)

OCIF濃度(ng/ml)	250	125	63	31	16	0
rOCIF(E)	0	0	3	62	80	100
nOCIF	0	0	27	27	75	100

nOCIFと同様にrOCIF(E)にも、 16ng/ml 以上の濃度で用量依存的な破骨細胞形成抑制活性が見られた。

ii) ストローマ細胞とマウス脾臓細胞の共培養系でのビタミン D_3 で誘導される破骨細胞形成の抑制

ビタミン D_3 で誘導されるストローマ細胞とマウス脾臓細胞の共培養系での破骨細胞形成の試験は、宇田川らの方法(Endocrinology, Vol. 125, p1805-1813, 1989)に従って行った。即ち、96ウェルマイクロプレートに $2 \times 10^{-8}\text{M}$ 活性型ビタミン D_3 、 $2 \times 10^{-7}\text{M}$ デキサメサゾン及び10%牛胎児血清を含む α -MEM培地(ギブコBRL社)で、連続的に希釈した精製rOCIF(E)、rOCIF(C)及びnOCIF

100 μ lを入れた。このウェルにマウス骨髄由来ストローマ細胞株 S T 2 細胞 (RIKEN Cell Bank-RCB0224) 5×10^3 個と生後約 8 週間の ddyマウス脾臓細胞 1×10^5 個を 100 μ l の 10%牛胎児血清を含む α -MEM 培地に懸濁させて播種し、5%CO₂、37℃、湿度 100%にて 5 日間培養した。培養 5 日後にリン酸塩緩衝生理食塩水で洗浄した後、エタノール/アセトン (1:1) 溶液で細胞を室温にて 1 分間固定し、破骨細胞形成を酸性ホスファターゼ活性測定キット (Acid Phosphatase, Leucocyte、カタログNo.387-A, シグマ社) を用いた染色で検出した。酒石酸存在下での酸性ホスファターゼ活性陽性細胞の減少を OCIF 活性とした。又、酸性ホスファターゼ活性陽性細胞数の減少率は実施例 16-i) に記載した方法に従って染色された細胞の色素を溶解させて算出した。rOCIF(E) と rOCIF(C) を用いて試験した結果を表 6 に、rOCIF(E) と nOCIF を用いて試験した結果を表 7 に、それぞれ示す。

第 6 表 ストローマ細胞とマウス脾臓細胞の共培養系での OCIF による破骨細胞形成抑制

OCIF濃度 (ng/ml)	50	25	13	6	0
rOCIF(E)	3	22	83	80	100
rOCIF(C)	13	19	70	96	100

第 7 表 ストローマ細胞とマウス脾臓細胞の共培養系での OCIF による破骨細胞形成抑制

OCIF濃度 (ng/ml)	250	63	16	0
rOCIF(E)	7	27	37	100
nOCIF	13	23	40	100

nOCIF と同様に rOCIF(E) 及び rOCIF(C) についても、6 ~ 16 ng/ml 以上の濃度で容量依存的な破骨細胞形成抑制活性が見られた。

iii) PTHで誘導される破骨細胞形成の抑制

PTHで誘導される破骨細胞形成の試験は、高橋らの方法 (Endocrinology, Vol.122, p1373-1382, 1988)に従って行った。即ち、96ウェルマイクロプレートに 2×10^{-8} M PTH及び10%牛胎児血清を含む α -MEM培地 (ギブコ社) で、125ng/mlから連続的に希釈したnOCIF及び精製rOCIF(E) 100 μ lを入れた。このウェルに生後約17日のマウス骨髓細胞 3×10^5 個を 100 μ l の10%牛胎児血清を含む α -MEM培地に懸濁させて播種し、5% CO₂、37℃、湿度100%にて5日間培養した。培養5日後にリン酸塩緩衝生理食塩水で洗浄した後エタノール/アセトン (1:1) 溶液で細胞を室温にて1分間固定し、破骨細胞形成を酸性ホスファターゼ活性測定キット (Acid Phosphatase, Leucocyte、カタログ No. 387-A, シグマ社) を用いた染色で検出した。酒石酸存在下での酸性ホスファターゼ活性陽性細胞の減少をOCIF活性とした。又、酸性ホスファターゼ活性陽性細胞数の減少率は実施例16-i)に記載した方法に従って染色された細胞の色素を溶解させて算出した。結果を表8に示す。

第8表 マウス骨髓細胞系でのOCIFによる破骨細胞形成抑制(PTH)

OCIF 濃度 (ng/ml)	125	63	31	16	8	0
rOCIF(E)	6	58	58	53	88	100
nOCIF	18	47	53	56	91	100

nOCIFと同様にrOCIF(E)についても、16ng/ml以上の濃度で容量依存的な破骨細胞形成抑制活性が見られた。

iv) IL-11で誘導される破骨細胞形成の抑制

IL-11で誘導される破骨細胞形成の試験は、田村らの方法 (Proc. Natl. Acad. Sci. USA, Vol.90, p11924-11928, 1993)に従って行った。即ち、96ウェルマイクロプレートに 20ng/ml IL-11及び10%牛胎児血清を含む α -MEM培地 (ギブコ BRL社製) で希釈したnOCIF及び精製rOCIF(E) 100 μ lを入れた。このウェルにマウス新生児頭蓋骨由来前脂肪細胞株 MC3T3-G2/PA6 細胞 (RIKEN Cell Bank-

RCB1127) 5×10^3 個と生後約 8 週間の ddy マウス 脾臓細胞 1×10^5 個を $100 \mu\text{l}$ の 10% 牛胎児血清を含む α -MEM 培地に懸濁させて播種し、5% CO_2 、 37°C 、湿度 100% にて 5 日間培養した。培養 5 日後にリン酸塩緩衝生理食塩水で洗浄した後エタノール/アセトン (1:1) 溶液で細胞を室温にて 1 分間固定し、破骨細胞形成を酸性ホスファターゼ活性測定キット (Acid Phosphatase, Leucocyte, カタログ No. 387-A, シグマ社) を用いた染色で検出した。酒石酸存在下での酸性ホスファターゼ活性陽性細胞数を計測し、その減少を OCIF 活性とした。結果を表 9 に示す。

第 9 表 IL-11 で誘導される酒石酸存在下での
酸性ホスファターゼ活性陽性細胞数

濃度 (ng/ml)	500	125	31	7.8	2.0	0.5	0
nOCIF	0	0	1	4	13	49	31
rOCIF(E)	0	0	1	3	10	37	31

nOCIF 及び rOCIF(E) とともに、 2ng/ml 以上の濃度で容量依存的に IL-11 で誘導される破骨細胞形成を抑制する活性が見られた。

このように種々の標的細胞を用いた破骨細胞形成の試験系において、OCIF はビタミン D_3 、PTH、及び IL-11 等の破骨細胞形成誘導因子による破骨細胞の形成をほぼ同じ濃度で抑制することが明らかになった。従って、OCIF はこのような様々な骨吸収促進物質で誘導される異なるタイプの骨量減少症の治療に、効果的に使用出来る可能性が示唆された。

〔実施例 17〕

モノマー型及びダイマー型 OCIF サンプルの調製

rOCIF(E) 及び rOCIF(C) それぞれ $100 \mu\text{g}$ を含むサンプルに、1/100 容量の 25% TFA (トリフルオロ酢酸) を加えた後、0.1% TFA を含む 30% アセトニトリルで平衡化した逆相カラム (PROTEIN-RP、 $2.0 \times 250\text{mm}$ 、ワイエムシー社) にか、50 分間でアセトニトリルを 55% にする直線勾配、流速 0.2ml/分 にて溶出を行い、各 OCIF ピークを分取した。得られたピーク画分を凍結乾燥すること

により、モノマー型OCIF及びダイマー型OCIFを得た。

〔実施例18〕

組み換え型OCIFの分子量測定

実施例3-vi)の方法で逆相カラムを用いて精製したモノマー型及びダイマー型nOCIFと実施例17記載の方法で精製したモノマー型及びダイマー型rOCIF約1 μ gを含むサンプルを減圧濃縮した。これらのサンプルにつき、実施例4の方法でSDS処理、SDS-ポリアクリルアミド電気泳動、及び銀染色を行った。非還元条件下及び還元条件下で電気泳動した結果を、図6及び図7にそれぞれ示す。

その結果、非還元条件下では、何れのモノマー型サンプルでも60kDの蛋白質バンドが検出され、又、何れのダイマー型サンプルでも120kDの蛋白質バンドが検出された。又、還元条件下では何れのサンプルでも約60kDの蛋白質バンドのみが検出された。従って、IMR-90細胞由来nOCIF、293/EBNA細胞由来組み換え型OCIF、及びCHO細胞由来組み換え型OCIFの各々のモノマー型とダイマー型の分子量はほぼ同一であることが示された。

〔実施例19〕

IMR-90細胞由来天然型OCIFと組み換え型OCIFのN-結合型糖鎖の除去と分子量測定

実施例3-vi)の方法で逆相カラムを用いて精製したモノマー型及びダイマー型nOCIFと実施例17記載の方法で精製したモノマー型及びダイマー型rOCIFの各々を約5 μ g含むサンプルを減圧濃縮した。これらのサンプルに100mM 2-メルカプトエタノールを加えた50mMリン塩緩衝液、pH8.6, 9.5 μ lを加えて溶解させ、更に250U/ml N-グリカナーゼ溶液(生化学工業社)0.5 μ lを加え37℃で一日放置した。これらのサンプルに2mM MEDTA、5%SDS、及び0.02%プロモフェノールブルーを含む20mM Tris-HCl, pH8.0, 10 μ lを加え、100℃で5分間加熱した。これらのサンプルの1 μ lを実施例4の方法でSDS-ポリアクリルアミド電気泳動した後、銀染色した。結果を図8に示す。

その結果、N-グリカナーゼ処理によりN-結合糖鎖を除去したOCIF蛋白

質の還元条件下での分子量は、いずれも約40kDであることが示された。糖鎖除去の処理を行っていないIMR-90細胞由来nOCIF、293/EBNA細胞由来rOCIF、及びCHO細胞由来rOCIFの各々の還元条件下での分子量はいずれも約60kDであることから、これらのOCIFはその分子内にN-結合糖鎖を含有する糖蛋白質であることが明らかになった。

〔実施例20〕

OCIF類縁体（バリエーション）cDNAのクローニング及び塩基配列の決定

実施例10及び11で示したように、純化したいいくつかの陽性ファージのひとつからpBKC MV（ストラタジーン社）にOCIFcDNA が挿入されたプラスミドpBKOCIFを持つ形質転換株を得たが、その際、他のいくつかの陽性ファージからも長さの異なるインサートが挿入されたプラスミドを持つ形質転換株が得られた。これらのプラスミドを持つ形質転換株を増殖させ、常法によりプラスミドを精製した。これらのインサートDNAの塩基配列をタックダイデオキシターミネーターサイクルシーケンシングキット（パーキンエルマー社）を用いて決定した。用いたプライマーはT3、T7プライマー（ストラタジーン社）及びOCIFcDNAの塩基配列に基づいて設計された合成プライマーを用いた。オリジナルタイプのOCIF以外に、OCIFバリエーションは全部で4種類（OCIF2、3、4、5）存在した。決定されたOCIF2cDNAの塩基配列を配列番号8にその配列から推定されるアミノ酸配列を配列番号9に示す。決定されたOCIF3 cDNAの塩基配列を配列番号10にその配列から推定されるアミノ酸配列を配列番号11に示す。決定されたOCIF4 cDNAの塩基配列を配列番号12にその配列から推定されるアミノ酸配列を配列番号13に示す。決定されたOCIF5 cDNAの塩基配列を配列番号14にその配列から推定されるアミノ酸配列を配列番号15に示す。これらのOCIFバリエーションの構造の特徴を、図9～12及び以下の記載をもって、簡単に説明する。

OCIF2

OCIFcDNAの塩基配列（配列番号6）の265番目のグアニンから285番目のグアニンまでの21bpの欠失があり、アミノ酸配列ではOCIFのアミノ酸配列（配列表配列番号5）の68番目のグルタミン酸（Glu）から74番目のグルタミン（G

1 n) までの7アミノ酸の欠失がある。

OCIF3

OCIFcDNAの塩基配列（配列番号6）の9番目のシチジンがグアニンに変換して、アミノ酸配列ではOCIFのアミノ酸配列（配列表配列番号5）の-19番目のアスパラギン（A s n）がリジン（L y s）に変わっている。但し、これはシグナル配列の中のアミノ酸置換であり、分泌されるOCIF3には影響しないと思われる。

OCIFcDNAの塩基配列（配列番号6）の872番目のグアニンから989番目のグアニンまでの117bpの欠失があり、アミノ酸配列ではOCIFのアミノ酸配列（配列表配列番号5）の270番目のスレオニン（T h r）から308番目のロイシン（L e u）までの39アミノ酸の欠失がある。

OCIF4

OCIFcDNAの塩基配列（配列番号6）の9番目のシチジンがグアニンに変換して、アミノ酸配列ではOCIFのアミノ酸配列（配列表配列番号5）の-19番目のアスパラギン（A s n）がリジン（L y s）に変わっている。又、22番目のグアニンがチミジンに変換して、アミノ酸配列ではOCIFのアミノ酸配列（配列表配列番号5）の-14番目のアラニン（A l a）がセリン（S e r）に変わっている。但し、これらはシグナル配列の中のアミノ酸置換であり、分泌されるOCIF4には影響しないと思われる。

OCIFcDNAの塩基配列（配列番号6）の400番目と401番目の間に約4kbのイントロン2の挿入があり、オープンリーディングフレームがその中で止まる。アミノ酸配列ではOCIFのアミノ酸配列（配列表配列番号5）の112番目のアラニン（A l a）の後に21アミノ酸からなる新規なアミノ酸配列が付加されている。

OCIF5

OCIFcDNAの塩基配列（配列番号6）の9番目のシチジンがグアニンに変換して、アミノ酸配列ではOCIFのアミノ酸配列（配列表配列番号5）の-19番目のアスパラギン（A s n）がリジン（L y s）に変わっている。但し、これはシグナル配列の中のアミノ酸置換であり、分泌されるOCIF5には影響しない

と思われる。

OCIFcDNAの塩基配列（配列番号6）の400番目と401番目の間に約1.8 kbのイントロン2の後半部分の挿入があり、オープンリーリングフレームがその中で止まる。アミノ酸配列ではOCIFのアミノ酸配列（配列表配列番号5）の112番目のアラニン（A1a）の後に12アミノ酸からなる新規なアミノ酸配列が付加されている。

〔実施例21〕

OCIF類縁体（バリエーション）の生産

i) OCIFバリエーションcDNAの発現プラスミドの作製

実施例20で得られたOCIFバリエーションcDNAのうち、OCIF2、3のcDNAがそれぞれ挿入されたプラスミドpBKOCIF2、pBKOCIF3を制限酵素XhoI及びBamHI（宝酒造社）で消化し、OCIF2及び3のcDNAをそれぞれ切り出し、アガロース電気泳動によって分離後、QIAEX ゲルエクストラクションキット（キアゲン社）を用いて精製した。これらのOCIF2及び3のcDNAを、あらかじめ制限酵素XhoI及びBamHI（宝酒造社）で消化しておいた発現プラスミドpCEP4（インヴィトロジェン社）に、ライゲーションキット Ver.2（宝酒造社）を用いて挿入し、大腸菌 DH5 α （ギブコBRL社）の形質転換を行った。

又、実施例20で得られたOCIFバリエーションcDNAのうち、OCIF4のcDNAを挿入されたプラスミドpBKOCIF4を制限酵素SpeI及びXhoI（宝酒造社）で消化し、アガロース電気泳動によって分離後、QIAEX ゲルエクストラクションキット（キアゲン社）を用いて精製した。このOCIF4のcDNAを、あらかじめ制限酵素NheI及びXhoI（宝酒造社）で消化しておいた発現プラスミドpCEP4（インヴィトロジェン社）に、ライゲーションキット Ver.2（宝酒造社）を用いて挿入し、大腸菌 DH5 α （ギブコBRL社）の形質転換を行った。

又、実施例20で得られたOCIFバリエーションcDNAのうち、OCIF5のcDNAを挿入されたプラスミドpBKOCIF5を制限酵素Hind III（宝酒造社）で消化し、OCIF5cDNAのコーディング領域の5'領域を切り出し、アガロース電気泳動によって分離後、QIAEX ゲルエクストラクションキット（キアゲン社）を用いて精

製した。実施例 13-i) で得られた OCIF 発現プラスミド pCEPOCIF を制限酵素 Hind III (宝酒造社) で消化し、OCIFcDNA のコーディング領域の 5' 領域を取り除き、pCEP プラスミドと OCIFcDNA の 3' 領域を含んだ DNA 断片 pCEPOCIF-3' をアガロース電気泳動によって分離後、QIAEX ゲルエクストラクションキット (キアゲン社) を用いて精製した。この OCIF5 cDNA の Hind III 断片を pCEPOCIF-3' にライゲーションキット Ver.2 (宝酒造社) を用いて挿入し、大腸菌 DH5 α (ギブコ BRL 社) の形質転換を行った。

得られた形質転換株を増殖させ、OCIF2, 3, 4, 5 の cDNA が挿入された発現プラスミド pCEPOCIF 2, 3, 4, 5 を、キアゲンカラム (キアゲン社) を用いて精製した。OCIF バリエント発現プラスミドをエタノールによって沈澱させた後、無菌蒸留水に溶解し以下の操作に用いた。

ii) OCIF バリエント cDNA のトランジェントな発現及びその活性の測定

実施例 21-i) で得られた OCIF バリエント発現プラスミド pCEPOCIF 2, 3, 4, 5 を用いて、実施例 13-ii) で述べた方法で OCIF バリエントをトランジェントに発現させ、それらの活性を調べた。その結果、これらの OCIF バリエントに弱い活性を認めた。

[実施例 22]

OCIF 変異体の作製

i) OCIF 変異体 cDNA サブクローニング用プラスミドベクターの作製

実施例 11 記載のプラスミドベクター 5 μ g を、制限酵素 BamHI 及び XhoI (宝酒造社) で切断した。切断した DNA を調製用アガロースゲル電気泳動に供した。OCIFcDNA 全長を含む約 1.6 キロベースペア (kb) の DNA 断片を単離し、QIAEX ゲルエクストラクションキット (キアゲン社) により精製し、20 μ l の滅菌蒸留水に溶解した DNA 溶液 1 を得た。次に、pBluescript IISK⁺ (ストラタジーン社) 3 μ g を制限酵素 BamHI 及び XhoI (宝酒造社) で切断した。切断した DNA を調製用アガロースゲル電気泳動に供した。約 3.0 kb の DNA 断片を単離し、QIAEX ゲルエクストラクションキット (キアゲン社) により精製し、20 μ l の滅菌蒸留水に溶解した DNA 溶液 2 を得た。1 μ l の DNA 溶液 2 と 4

μ l の DNA 溶液 1 を混合し、5 μ l の DNA ライゲーションキット ver.2 1 液 (宝酒造社) を添加し混合後、16℃ で 30 分間保温し、ライゲーション反応を行った。尚、以下のライゲーション反応は全て 16℃ 30 分の保温条件で行った。

このライゲーション反応液を用い、以下の条件で大腸菌の形質転換を行った。尚、以後大腸菌の形質転換は以下の条件で行った。このライゲーション反応液 5 μ l と大腸菌 DH5 α コンピテント細胞 (ギブコ BRL 社) 100 μ l とを 15ml 用滅菌チューブ (岩城ガラス社) 中で混合し、氷水中 30 分放置した。42℃ 45 秒保温後、250 μ l の L 培地 (1% トリプトン、0.5% イーストエキストラクト、1% NaCl) を添加し攪拌しながら 37℃ で培養した。50 μ l の菌液を 50 μ g/ml アンピシリンを含む 2ml の L 寒天培地上にスプレッドした。37℃ で一晩培養し、生育してきたコロニー 6 種を 2ml の L アンピシリン液体培地でさらに一晩培養し、各株が持つプラスミドの構造を調べた。pBluescript IISK⁺ の BamHI XhoI 切断部位に OCIFcDNA 全長を含む約 1.6kb の DNA 断片が挿入された構造を持つプラスミド (以後 pSK⁺-OCIF と呼ぶ) を得た。

ii) Cys を Ser に置換した変異体の作製

(1) 変異の導入

配列表配列番号 4 に記載のアミノ酸配列中、174、181、256、298 及び 379 番の Cys 残基を Ser 残基に置換した変異体を作製した。174Cys を Ser に置換した変異体を OCIF-C19S、181Cys を Ser に置換した変異体を OCIF-C20S、256Cys を Ser に置換した変異体を OCIF-C21S、298Cys を Ser に置換した変異体を OCIF-C22S、379Cys を Ser に置換した変異体を OCIF-C23S2 と、それぞれ名付けた。変異体作製のためにまず、各 Cys 残基をコードする塩基配列を Ser 残基をコードする塩基配列に置換した。変異導入は二段階の PCR (polymerase chain reaction) により行った。以後、二段階 PCR 反応と呼ぶ。第一段階は 2 つの PCR 反応より成る (PCR1 及び PCR2)。

P C R 1 反応液

10X Ex Taq バッファー (宝酒造社)	1 0	μ l
2.5 mM dNTP 溶液	8	μ l
実施例 1 1 記載のプラスミドベクター (8ng/ml)	2	μ l
滅菌蒸留水	7 3. 5	μ l
2 0 μ M プライマー 1	5	μ l
1 0 0 μ M プライマー 2 (変異導入用)	1	μ l
Ex Taq (宝酒造社)	0. 5	μ l

P C R 2 反応液

10X Ex Taq バッファー (宝酒造社)	1 0	μ l
2.5 mM dNTP 溶液	8	μ l
実施例 1 1 記載のプラスミドベクター (8ng/ml)	2	μ l
滅菌蒸留水	7 3. 5	μ l
2 0 μ M プライマー 3	5	μ l
1 0 0 μ M プライマー 4 (変異導入用)	1	μ l
Ex Taq (宝酒造社)	0. 5	μ l

各変異導入時には、プライマーの種類だけを変え、他の反応組成は同一とした。各反応で用いたプライマーを表10に、その配列を配列表配列番号20、23、27、30～40に示す。P C R 1 反応液及びP C R 2 反応液をそれぞれ別の微量遠心チューブに入れ混合後、以下の条件でP C Rを行った。97℃で3分処理後、95℃1分、55℃1分、72℃3分の3段階の反応を25回繰り返したのち、70℃5分保温した。反応液の一部をアガロース電気泳動に供し、目的の長さのDNA断片が合成されていることを確認した。第一段階P C R反応終了後、アミコンマイクロコン (アミコン社) により反応液からプライマーを除去し、滅菌蒸留水により最終液量を50 μ l に調製し、得られたDNA断片を用いさらに第2段階P C R反応 (P C R 3) を行った。

P C R 3 反 応 液

10X Ex Taqバッファー (宝酒造社)	1 0	μ l
2.5 mM dNTP 溶液	8	μ l
P C R 1 により得られたDNA断片	5	μ l
P C R 2 により得られたDNA断片	5	μ l
滅菌蒸留水	6 1 . 5	μ l
20 μ M プライマー 1	5	μ l
20 μ M プライマー 3	5	μ l
Ex Taq (宝酒造社)	0 . 5	μ l

第 1 0 表

変異体名	プライマー-1	プライマー-2	プライマー-3	プライマー-4
OCIF-C19S	IF 10	C19SR	IF 3	C19SF
OCIF-C20S	IF 10	C20SR	IF 3	C20SF
OCIF-C21S	IF 10	C21SR	IF 3	C21SF
OCIF-C22S	IF 10	C22SR	IF 14	C22SF
OCIF-C23S	IF 6	C23SR	IF 14	C23SF

上記の溶液を微量遠心チューブに入れ混合後、P C R 1、P C R 2 と同一の条件でP C Rを行った。反応液の一部をアガロース（1%或いは1.5%）電気泳動に供し、目的の長さのDNA断片が合成されていることを確認した。P C Rにより得られたDNAをエタノールにより沈殿させ、真空中で乾燥させ、40 μ l の滅菌蒸留水に溶解した。C19S変異DNA断片を含む溶液を溶液A、C20S変異DNA断片を含む溶液を溶液B、C21S変異DNA断片を含む溶液を溶液C、C22S変異DNA断片を含む溶液を溶液D、C23S変異DNA断片を含む溶液を溶液Eと名付けた。

溶液A 20 μ l 中のDNA断片を制限酵素NdeI及びSphI（宝酒造社）により切断した。調製用電気泳動により約400bp のDNA断片を分離・精製し20 μ l の蒸留

水に溶解した(DNA溶液3)。次に、2 μ g のpSK⁺-OCIF を制限酵素NdeI及びSphI(宝酒造社)により切断し、調製用電気泳動により約4.2kbのDNA断片を分離・精製し20 μ lの滅菌蒸留水に溶解した(DNA溶液4)。2 μ lのDNA溶液3と3 μ lのDNA溶液4を混合し、さらにDNAライゲーションキットver.2 1液5 μ lを添加しライゲーション反応を行った。反応後のライゲーション溶液5 μ lを用い、大腸菌DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA構造の解析により目的のプラスミドDNAを持つ株を選びだした。DNA構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミドDNAをpSK-OCIF-C19Sと名付けた。

溶液B 20 μ l中のC20S変異DNA断片を制限酵素NdeI及びSphI(宝酒造社)により切断した。調製用電気泳動により約400bpのDNA断片を分離・精製し20 μ lの蒸留水に溶解した(DNA溶液5)。2 μ lのDNA溶液5と3 μ lのDNA溶液4を混合し、さらにDNAライゲーションキットver.2 1液5 μ lを添加しライゲーション反応を行った。反応後のライゲーション溶液5 μ lを用い、大腸菌DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA構造の解析により目的のプラスミドDNAを持つ株を選びだした。DNA構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミドDNAをpSK-OCIF-C20Sと名付けた。

溶液C 20 μ l中のDNA断片を制限酵素NdeI及びSphI(宝酒造社)により切断した。調製用電気泳動により約400bpのDNA断片を分離・精製し20 μ lの蒸留水に溶解した(DNA溶液6)。2 μ lのDNA溶液6と3 μ lのDNA溶液4を混合し、さらにDNAライゲーションキットver.2 1液5 μ lを添加しライゲーション反応を行った。反応後のライゲーション溶液5 μ lを用い、大腸菌DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA構造の解析により目的のプラスミドDNAを持つ株を選びだした。DNA構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミドDNAをpSK-OCIF-C21Sと名付けた。

溶液 D 20 μ l 中の DNA 断片を制限酵素 NdeI 及び BstPI (宝酒造社) により切断した。調製用電気泳動により約 600bp の DNA 断片を分離・精製し 20 μ l の蒸留水に溶解した (DNA 溶液 7)。次に、2 μ g の pSK⁺-OCIF を制限酵素 NdeI 及び BstPI (宝酒造社) により切断し、調製用電気泳動により約 4.0kb の DNA 断片を分離・精製し 20 μ l の蒸留水に溶解した (DNA 溶液 8)。2 μ l の DNA 溶液 7 と 3 μ l の DNA 溶液 8 を混合し、さらに DNA ライゲーションキット ver.2 1 液 5 μ l を添加しライゲーション反応を行った。反応後のライゲーション溶液 5 μ l を用い、大腸菌 DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA 構造の解析により目的のプラスミド DNA を持つ株を選び出した。DNA 構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミド DNA を pSK-OCIF-C22S と名付けた。

溶液 E 20 μ l 中の DNA 断片を制限酵素 BstPI 及び EcoRV (宝酒造社) により切断した。調製用電気泳動により約 120bp の DNA 断片を分離・精製し 20 μ l の滅菌蒸留水に溶解した (DNA 溶液 9)。次に、2 μ g の pSK⁺-OCIF を制限酵素 BstEII 及び EcoRV (宝酒造社) により切断し、調製用電気泳動により約 4.5kb の DNA 断片を分離・精製し 20 μ l の蒸留水に溶解した (DNA 溶液 10)。2 μ l の DNA 溶液 9 と 3 μ l の DNA 溶液 10 を混合し、さらに DNA ライゲーションキット ver.2 1 液 5 μ l を添加しライゲーション反応を行った。反応後のライゲーション溶液 5 μ l を用い、大腸菌 DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA 構造の解析により目的のプラスミド DNA を持つ株を選び出した。DNA 構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミド DNA を pSK-OCIF-C23S と名付けた。

(2) 変異体発現ベクターの構築

得られた目的のプラスミド DNA (pSK-OCIF-C19S, pSK-OCIF-C20S, pSK-OCIF-C21S, pSK-OCIF-C22S, pSK-OCIF-C23S) を制限酵素 BamHI 及び XhoI (宝酒造社) で切断し、OCIFcDNA 全長を含む約 1.6kb の DNA 断片 (目的の変異も含む) を分離・

精製し、滅菌蒸留水 20 μ l に溶解した。それぞれ C19SDNA 溶液、C20SDNA 溶液、C21SDNA 溶液、C22SDNA 溶液、C23SDNA 溶液と名付けた。次に、発現ベクター pCEP4 (インヴィトロジェン社) 5 μ g を制限酵素 BamHI 及び XhoI (宝酒造社) で切断し、約 10 kb の DNA を分離・精製し滅菌蒸留水 40 μ l に溶解した (pCEP4DNA 溶液)。pCEP4DNA 溶液 1 μ l と各 6 μ l の C19SDNA 溶液、C20SDNA 溶液、C21SDNA 溶液、C22SDNA 溶液、C23SDNA 溶液を別々に混合し、各混合液に 7 μ l の DNA ライゲーションキット Ver.2 I 液を添加し、ライゲーション反応を行った。反応終了後、7 μ l の反応液を用い、大腸菌 DH5 α コンピテント細胞液 100ml を形質転換した。得られたアンピシリン耐性形質転換細胞から、pCEP4 の XhoI、BamHI 部位に約 1.6kb の各 DNA 断片が挿入された目的の構造のプラスミド DNA を持つ株計 5 種を選びだし、それぞれ、pCEP4-OCIF-C19S, pCEP4-OCIF-C20S, pCEP4-OCIF-C21S, pCEP4-OCIF-C22S, pCEP4-OCIF-C23S と名付けた。

ii) ドメイン欠失変異体の作製

(1) ドメイン欠失変異の導入

配列番号 4 に記載したアミノ酸中、2 番の Thr から 42 番の Ala まで、43 番の Pro から 84 番の Cys まで、85 番の Glu から 122 番の Lys まで、123 番の Arg から 164 番の Cys まで、177 番の Asp から 251 番の Gln まで、253 番の Ile から 326 番の His までを、それぞれ欠失させた変異体を作製した。2 番の Thr から 42 番の Ala までを欠失させた変異体を OCIF-DCR1、43 番の Pro から 84 番の Cys までを欠失させた変異体を OCIF-DCR2、85 番の Glu から 122 番の Lys までを欠失させた変異体を OCIF-DCR3、123 番の Arg から 164 番の Cys までを欠失させた変異体を OCIF-DCR4、177 番の Asp から 251 番の Gln までを欠失させた変異体を OCIF-DDD1、253 番 Ile から 326 番の His までを欠失させた変異体を OCIF-DDD2 と、それぞれ名付けた。ドメイン欠失変異の導入も、実施例 22-ii) に記載の二段階 PCR 法によって行った。各変異導入反応時に用いたプライマーを表 11 に、その配列を配列表配列番号 19、25、40~53、及び 54 に示す。

第 1 1 表

変異体名	プライマー-1	プライマー-2	プライマー-3	プライマー-4
OCIF-DCR1	XhoI F	DCR1R	IF 2	DCR1F
OCIF-DCR2	XhoI F	DCR2R	IF 2	DCR2F
OCIF-DCR3	XhoI F	DCR3R	IF 2	DCR3F
OCIF-DCR4	XhoI F	DCR4R	IF 16	DCR4F
OCIF-DDD1	IF 8	DDD1R	IF 14	DDD1F
OCIF-DDD2	IF 8	DDD2R	IF 14	DDD2F

P C Rにより得られたDNAをエタノールにより沈殿させ真空中で乾燥させ、40 μ lの滅菌蒸留水に溶解した。DCR1変異DNA断片を含む溶液を溶液F、DCR2変異DNA断片を含む溶液を溶液G、DCR3変異DNA断片を含む溶液を溶液H、DCR4変異DNA断片を含む溶液を溶液I、DDD1変異DNA断片を含む溶液を溶液J、DDD2変異DNA断片を含む溶液を溶液Kと名付けた。

溶液F 20 μ l 中のDNA断片を制限酵素NdeI及びXhoI（宝酒造社）により切断した。調製用電気泳動により約500bp のDNA断片を分離・精製し20 μ l の滅菌蒸留水に溶解した（DNA溶液11）。次に、2 μ g のpSK⁺-OCIFを制限酵素NdeI及びXhoI（宝酒造社）により切断し、調製用電気泳動により約4.0kb のDNA断片を分離・精製し20 μ l の滅菌蒸留水に溶解した（DNA溶液12）。2 μ l のDNA溶液11と3 μ l のDNA溶液12を混合し、さらにDNAライゲーションキットver.2 I液5 μ l を添加しライゲーション反応を行った。反応後のライゲーション溶液5 μ l を用い、大腸菌DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA構造の解析により、OCIFcDNAに目的の変異の導入されたプラスミドDNAを持つ株を選び出した。DNA構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミドDNAをpSK-OCIF-DCR1と名付けた。溶液G 20 μ l 中のDNA断片を制限酵素NdeI及びXhoI（宝酒造社）により切断した。調製用電気泳動により約500bp のDNA断片を分離・精製し20 μ l の滅菌蒸留水に溶解した（DNA溶液13）。2 μ l のDNA溶液13と3 μ l のDNA溶液12を混合し、さらにD

NAライゲーションキットver.2 1液を5 μ l 添加し、ライゲーション反応を行った。反応後のライゲーション溶液5 μ l を用い、大腸菌DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA構造の解析により目的のプラスミドDNAを持つ株を選びだした。DNA構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミドDNAをpSK-OCIF-DCR2 と名付けた。

溶液H 20 μ l 中のDNA断片を制限酵素NdeI及びXhoI（宝酒造社）により切断した。調製用電気泳動により約500bp のDNA断片を分離・精製し20 μ l の滅菌蒸留水に溶解した（DNA溶液14）。2 μ l のDNA溶液14と3 μ l のDNA溶液12を混合し、さらにDNAライゲーションキットver.2 1液を5 μ l 添加し、ライゲーション反応を行った。反応後のライゲーション溶液5 μ l を用い、大腸菌DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA構造の解析により、OCIF c DNAに目的の変異の導入されたプラスミドDNAを持つ株を選びだした。DNA構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミドDNAをpSK-OCIF-DCR3 と名付けた。

溶液I 20 μ l 中のDNA断片を制限酵素XhoI及びSphI（宝酒造社）により切断した。調製用電気泳動により約900bp のDNA断片を分離・精製し20 μ l の滅菌蒸留水に溶解した（DNA溶液15）。次に、2 μ g のpSK-OCIFを制限酵素XhoI及びSphI（宝酒造社）により切断し、調製用電気泳動により約3.6kb のDNA断片を分離・精製し20 μ l の滅菌蒸留水に溶解した（DNA溶液16）。2 μ l のDNA溶液15と3 μ l のDNA溶液16を混合し、さらにDNAライゲーションキットver.2 1液5 μ l を添加し、ライゲーション反応を行った。反応後のライゲーション溶液5 μ l を用い、大腸菌DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA構造の解析により目的のプラスミドDNAを持つ株を選びだした。DNA構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミドDNAをpSK-OCIF-DCR4 と名付けた。

溶液 J 20 μ l 中の DNA 断片を制限酵素 BstPI 及び NdeI (宝酒造社) により切断した。調製用電気泳動により約 400bp の DNA 断片を分離・精製し 20 μ l の滅菌蒸留水に溶解した (DNA 溶液 17)。2 μ l の DNA 溶液 17 と 3 μ l の DNA 溶液 8 を混合し、さらに DNA ライゲーションキット ver. 2 1 液を 5 μ l 添加し、ライゲーション反応を行った。反応後のライゲーション溶液 5 μ l を用い、大腸菌 DH 5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA 構造の解析により目的のプラスミド DNA を持つ株を選び出した。DNA 構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミド DNA を pSK-OCIF-DDD1 と名付けた。

溶液 K 20 μ l 中の DNA 断片を制限酵素 BstPI 及び NdeI (宝酒造社) により切断した。調製用電気泳動により約 400bp の DNA 断片を分離・精製し 20 μ l の滅菌蒸留水に溶解した (DNA 溶液 18)。2 μ l の DNA 溶液 18 と 3 μ l の DNA 溶液 8 を混合し、さらに DNA ライゲーションキット ver. 2 1 液を 5 μ l 添加し、ライゲーション反応を行った。反応後のライゲーション溶液 5 μ l を用い、大腸菌 DH 5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA 構造の解析により目的のプラスミド DNA を持つ株を選び出した。DNA 構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミド DNA を pSK-OCIF-DDD2 と名付けた。

(2) 変異体発現ベクターの構築

得られた目的のプラスミド DNA (pSK-OCIF-DCR1, pSK-OCIF-DCR2, pSK-OCIF-XR3, pSK-OCIF-DCR4, pSK-OCIF-DDD1, pSK-OCIF-DDD2) を制限酵素 BamHI 及び XhoI (宝酒造社) で切断し OCIFcDNA 全長を含む約 1.4-1.5 kb の DNA 断片 (目的の変異も含む) を分離・精製し、滅菌蒸留水 20 μ l に溶解した。それぞれを DCR1DNA 溶液、DCR2DNA 溶液、DCR3DNA 溶液、DCR4DNA 溶液、DDD1DNA 溶液、DDD2DNA 溶液と名付けた。実施例 22-ii) に記載の pCEP4 DNA 溶液 1 μ l と各 6 μ l の DCR1DNA 溶液、DCR2DNA 溶液、DCR3DNA 溶液、DCR4DNA 溶液、DDD1DNA 溶液、DDD2DNA 溶液を別々に混合し、各混合液に 7 μ l の DNA ライゲーションバッファーを添加し、ライゲーション反応を行った。反応終了後、7 μ l の反応液を用い、大腸

菌DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞からpCEP4 BamHI XhoI部位に各1.4-1.5kb断片が挿入された構造のプラスミドDNAを持つ株計6種を選びだした。目的の構造を持つプラスミドをそれぞれpCEP4-OCIF-DCR1、pCEP4-OCIF-DCR2、pCEP4-OCIF-DCR3、pCEP4-OCIF-DCR4、pCEP4-OCIF-DDD1、pCEP4-OCIF-DDD2と名付けた。

iii) C末端ドメイン欠失変異体の作製

(1) C末端ドメイン欠失変異の導入

配列番号4に記載したアミノ酸中、379番のCysと380番のLeu、331番のSerから380番のLeuまで、252番のAspから380番のLeuまで、177番のAspから380番のLeuまで、123番のArgから380番のLeuまで、86番のCysから380番のLeuまでを、それぞれ欠失させた変異体を作製した。379番のCysと380番のLeuを欠失させた変異体をOCIF-CL、331番のSerから380番のLeuまでを欠失させた変異体をOCIF-CC、252番のAspから380番のLeuまでを欠失させた変異体をOCIF-CDD2、177番のAspから380番のLeuまでを欠失させた変異体をOCIF-CDD1、123番のArgから380番のLeuまでを欠失させた変異体をOCIF-CCR4、86番のCysから380番のLeuまでを欠失させた変異体をOCIF-CCR3と、それぞれ名付けた。

変異体OCIF-CLの作製用の変異導入は、実施例22-ii)に記載の二段階PCR法によって行った。変異導入反応時に用いたプライマーを表12に、その塩基配列を配列表配列番号23、40、55及び56に示す。PCRにより得られたDNAをエタノールにより沈殿させ、真空中で乾燥させ、40 μ lの滅菌蒸留水に溶解した(溶液L)。

溶液L 20 μ l中のDNA断片を制限酵素BstPI及びEcoRV(宝酒造社)により切断した。調製用電気泳動により約100bpのDNA断片を分離・精製し20 μ lの滅菌蒸留水に溶解した(DNA溶液19)。次に、2 μ lのDNA溶液9と3 μ lの実施例22-ii)記載のDNA溶液10を混合し、さらにDNAライゲーションキットver.2 I液を5 μ l添加し、ライゲーション反応を行った。反応後のライゲーション溶液5 μ lを用い、大腸菌DH5 α を形質転換した。得られたアンピシ

リン耐性形質転換細胞から、DNA構造の解析により目的のプラスミドDNAを持つ株を選びだした。DNA構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミドDNAをpSK-OCIF-CLと名付けた。変異体OCIF-CC、変異体OCIF-CDD2、変異体OCIF-CDD1、変異体をOCIF-CCR4、変異体OCIF-CCR3 作製用の変異導入には、一段階のPCR法を用いた。以下に反応条件を示す。

C末端ドメイン欠失変異導入用PCR 反応液

10X Ex Taq バッファー (宝酒造社)	10	μ l
2.5 mM dNTP 溶液	8	μ l
実施例11記載のプラスミドベクター (8ng/ml)	2	μ l
滅菌蒸留水	73.5	μ l
20 μ M プライマー OCIF Xho F	5	μ l
100 μ M 変異導入用プライマー	1	μ l
Ex Taq (宝酒造社)	0.5	μ l

第12表

変異体名	プライマー1	プライマー2	プライマー3	プライマー4
OCIF-CL	IF 6	CL R	IF 14	CL F

各変異導入時には、プライマーの種類だけを変え、他の反応組成は同一とした。各反応での変異導入用プライマーを表13に、その配列を配列表配列番号57～61に示す。PCR反応液を微量遠心チューブに入れ混合後、以下の条件でPCRを行った。97℃で3分処理後、95℃30秒、50℃30秒、70℃3分の3段階の反応を25回繰り返したのち、70℃5分保温した。反応液の一部をアガロース電気泳動に供し、目的の長さのDNA断片が合成されていることを確認した。反応液からアミコン・マイクロコンによりプライマーを除去し、DNAをエタノールにより沈殿させ

真空中で乾燥させ、40 μ l の滅菌蒸留水に溶解した。各変異DNA断片を含む溶液20 μ l 中のDNA断片を制限酵素XhoI及びBamHIによりDNAを切断した。酵素切断終了後、DNAをエタノールにより沈殿させ真空中で乾燥させ、20 μ l の滅菌蒸留水に溶解した。溶液をそれぞれCCDNA 溶液、CDD2DNA 溶液、CDD1DNA 溶液、CCR4DNA 溶液、CCR3DNA 溶液と名付けた。

第13表

変異体名	変異導入用プライマー
OCIF-CC	CC R
OCIF-CDD2	CDD2 R
OCIF-CDD1	CDD1 R
OCIF-CCR4	CCR4 R
OCIF-CCR3	CCR3 R

(2) 変異体発現ベクターの構築

pSK-OCIF-CL を制限酵素BamHI 及びXhoI (宝酒造社) で切断し、OCIFcDNAを含む約1.5 kbのDNA断片 (目的の変異も含む) を分離・精製し、滅菌蒸留水20 μ l に溶解した (CLDNA 溶液)。実施例22-ii) に記載のpCEP4 DNA 溶液1 μ l と各6 μ l のCLDNA 溶液、CCDNA 溶液、CDD2DNA 溶液、CDD1DNA 溶液、CCR4DNA 溶液、CCR3DNA 溶液を別々に混合し、7 μ l のDNAライゲーションキット Ver.2 I液を添加し、ライゲーション反応を行った。反応終了後、7 μ l の反応液を用い、大腸菌DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から目的の変異を持つOCIFcDNA断片がpCEP4 のXhoI-BamHI部位に挿入された構造のプラスミドDNAを持つ株計6種を選び出した。目的の構造を持つプラスミドをそれぞれ、pCEP4-OCIF-CL, pCEP4-OCIF-CC, pCEP4-OCIF-CDD2, pCEP4-OCIF-CDD1, pCEP4-OCIF-CCR4, pCEP4-OCIF-CCR3と名付けた。

iv) C末端欠失変異体の作製

(1) C末端欠失変異の導入

配列番号4に記載したアミノ酸中、371番Glnから380番Leuまでを欠失させLeu-Valの2残基を付加した変異体(OCIF-CBst)、298番Cysから380番Leuまでを欠失させSer-Leu-Aspの残基を付加した変異体(OCIF-CSph)、167番Asnから380番Leuまでを欠失させた変異体(OCIF-CBsp)、62番Cysから380番Leuまでを欠失させLeu-Valの2残基を付加した変異体(OCIF-CPst)を作製した。各2 μ gのpSK'-OCIFを制限酵素BstPI、SphI、PstI(宝酒造社)、及びBspEI(ニューイングランドバイオラボ社)で切断し、フェノール処理、エタノール沈殿によりDNAを精製し、10 μ lの滅菌蒸留水に溶解した。各2 μ lの溶液を用いDNAブランチングキット(宝酒造社)により各DNAの末端を平滑化した(最終容量5 μ l)。この反応液に、アンバーコドンを含むXbaIリンカー(5'-CTAGTCTAGACTAG-3')1 μ g(1 μ l)と、6 μ lのDNAライゲーションキットver.2 I液を添加し、ライゲーション反応を行った。反応後のライゲーション溶液6 μ lを用い、大腸菌DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から、DNA構造の解析により目的のプラスミドDNAを持つ株を選びだした。DNA構造は、制限酵素切断により得られる断片の長さの測定及び塩基配列の決定により解析した。得られた目的のプラスミドDNAをpSK-OCIF-CBst、pSK-OCIF-CSph、pSK-OCIF-CBsp、pSK-OCIF-CPstと名付けた。

(2) 変異体発現ベクターの構築

得られたプラスミドDNA(pSK-OCIF-CBst、pSK-OCIF-CSph、pSK-OCIF-CBsp、pSK-OCIF-CPst)を制限酵素BamHI及びXhoI(宝酒造社)で切断し、OCIFcDNA全長を含む約1.5キロベースペア(kb)のDNA断片(目的の変異も含む)を分離・精製し、滅菌蒸留水20 μ lに溶解した(それぞれCBstDNA溶液、CSphDNA溶液、CBspDNA溶液、CPstDNA溶液と名付けた)。実施例22-ii)に記載のpCEP4 DNA溶液1 μ lと各6 μ lのCBstDNA溶液、CSphDNA溶液、CBspDNA溶液、CPstDNA溶液を別々に混合し、各混合液に7 μ lのDNAライゲーションキット Ver.2 I液を添加し、ライゲーション反応を行った。反応終了後、7 μ lの反応液を用い、大腸菌DH5 α を形質転換した。得られたアンピシリン耐性形質転換細胞から目的の変異を持つOCIFcDNA断片がpCEP4のXhoI BamHI部位間に挿入された構造のプ

ラスミドDNAを持つ株計5種を選びだした。目的の構造を持つプラスミドをそれぞれ、pCEP4-OCIF-CBst, pCEP4-OCIF-CSph, pCEP4-OCIF-CBsp, pCEP4-OCIF-CPstと名付けた。

v) 変異体発現ベクターの調製

変異体発現ベクターを持つ大腸菌(計21種類)を増殖させ、各種変異体発現ベクターをキアゲンカラム(キアゲン社)を用いて精製した。各発現ベクターはエタノールによって沈殿させた後、滅菌蒸留水に溶解し以下の操作に用いた。

vi) 変異体cDNAのトランジェントな発現及びその活性の測定

実施例22-v)で精製した各種OCIF変異体発現プラスミドを用い、実施例13の方法に従いOCIF変異体を発現させた。以下に変更した点のみを記す。DNA導入には24ウェルプレートを用いた。2×10⁵個の293/EBNA細胞を10%牛胎児血清を含むIMDM培地を用いて各ウェルに植え込んだ。DNA導入の際用いた変異体発現ベクターとリポフェクタミンの量は、それぞれ1μg及び4μlであった。OPTI-MEM培地(ギブコBRL社)で希釈し最終容量を0.5mlとした。変異体発現ベクターとリポフェクタミンの混合液を細胞に添加し、24時間37℃でCO₂インキュベーター中で培養した後混合液を除去し、0.5mlのEx-cell 301培地(JSR社)を加え、さらに48時間37℃でCO₂インキュベーター中で培養した。培地を回収し、これを変異体活性測定用サンプルとした。得られた各変異体の塩基配列を配列表配列番号83~103に、その配列から推定されるアミノ酸配列を配列表配列番号62~82に、それぞれ示す。OCIFの活性測定は実施例13に従った。また、実施例24に記載のEIA法により、OCIFの抗原量を定量した。表14に未改変OCIFと比較した抗原量当たりの活性を示す。

第 1 4 表

変異体の名称	活性
未改変OCIF	++
OCIF-C19S	+
OCIF-C20S	±
OCIF-C21S	±
OCIF-C22S	+
OCIF-C23S	++
OCIF-DCR1	±
OCIF-DCR2	±
OCIF-DCR3	±
OCIF-DCR4	±
OCIF-DDD1	+
OCIF-DDD2	±
OCIF-CL	++
OCIF-CC	++
OCIF-CDD2	++
OCIF-CDD1	+
OCIF-CCR4	±
OCIF-CCR3	±
OCIF-CBst	++
OCIF-CSph	++
OCIF-CBsp	±
OCIF-CPst	±

(表中、++は抗原量当たりの活性が未改変OCIFの活性の50%を超える、+は10%~50%、±は10%未満又は抗原量が正確に測定できないことをそれぞれ示す)

vi) ウェスタンブロッティング解析

活性測定に用いたサンプルの10 μ l をウェスタンブロット解析に供した。サンプル10 μ l に10 μ l のSDS-PAGE用サンプルバッファー(0.5M Tris-HCl、20%グリセロール、4%SDS、20 μ g/mlブロムフェノールブルー(pH 6.8))を加え、100℃で3分煮沸し非還元状態で10%SDSポリアクリルアミド電気泳動を行った。泳動終了後、セミドライブロッティング装置(パイオラッド社)によりPVDFメンブレン(ProBlott®、パーキンエルマー社)に蛋白質をブロッティングした。そのメンブレンをブロッキング後、実施例24に記載のEIA用西洋ワサビパーオキシダーゼ標識抗OCIF抗体とともに、37℃で2時間保温した。洗浄後ECLシステム(アマシャム社)により抗OCIF抗体に結合する蛋白質を検出した。OCIFでは、約120キロダルトン(kD)及び60kDのバンドが検出された。一方、OCIF-C23S、OCIF-CL、OCIF-CCでは、ほとんど60kDのバンドのみが検出された。また、OCIF-CDD2及びOCIF-CDD1ではそれぞれ約40-50 kD及び30-40 kDのバンドが主要なバンドとして検出された。以上の結果より、OCIFでは、配列表配列番号4のアミノ酸配列にける379番目のCys残基が二量体形成に係わっていること、単量体でも活性を保持していること、及び177番Aspから380番Leuまでの残基を欠失させても活性を保持していることが明らかとなった。

〔実施例23〕ヒトOCIFゲノムDNAの分離I) ヒトゲノムDNAライブラリーのスクリーニング

ヒト胎盤の染色体DNAと λ FIX IIベクターを用いて作製されたゲノム・ライブラリーをストラタジーン社から購入し、これをOCIFcDNAをプローブとしてスクリーニングした。スクリーニングは、基本的にはゲノム・ライブラリーに添付されているプロトコールに従って実施したが、ファージ、大腸菌、DNAを扱う一般的方法はMolecular Cloning: A Laboratory Manualに従って行った。

購入したゲノムDNAライブラリーのタイターを検定したのち、 1×10^6 pfuのファージを大腸菌XL1-Blue MRAに感染させ、20枚のプレート(9 \times 13cm)にプレ

ート当たり 9 ml のトップ・アガロースとともに蒔いた。プレートを一晩 37℃ でインキュベートしたのち、Hybond-N ナイロン膜（アマシャム社）をアガープレート上に乗せてファージを転写した。ファージの転写したナイロン膜を 1.5 M NaCl / 0.5 M NaOH 溶液で湿らせた濾紙上に 1 分間乗せ、その後 1 M Tris-HCl (pH 7.5) と 1.5 M NaCl / 0.5 M Tris-HCl (pH 7.5) でそれぞれ 1 分ずつ処理して中和したのち、最後に 2 X SSC で湿らせた濾紙の上に移した。その後、このナイロン膜にストラタリンカー（ストラタジーン社）を用いて 1200 マイクロジュールの UV を照射することによってファージ DNA を膜に固定した。次に、このナイロン膜をラビッドハイブリダイゼーション・バッファー（アマシャム社）に浸漬してプレハイブリダイゼーションを行った。1 時間のプレハイブリダイゼーションの後、³²P 標識した OCIFcDNA を加え、65℃ にて一夜ハイブリダイゼーションを行った。この cDNA プロブは、実施例 11 で得られた 1.6 kb の OCIFcDNA を有するプラスミド pBKOCIF を、制限酵素 BamHI 及び XhoI を用いて切断し、OCIFcDNA をアガロースゲル電気泳動によって単離したのち、この OCIFcDNA をメガプライム DNA ラベリングシステム（アマシャム社）を用いて ³²P で標識することによって作製した。標識は、ラベリングシステムに添付されたプロトコールに従って行った。ハイブリダイゼーションには、ハイブリダイゼーション・バッファー 1 ml 当たりおよそ 5×10^5 cpm のプロブを使用した。ハイブリダイゼーションの後、ナイロン膜を室温にて 2 X SSC で 5 分間洗浄し、その後 65℃ において 0.5 X SSC / 0.1% SDS で 4 回、それぞれ 20 分ずつ洗浄した。4 回目の洗浄ののちナイロン膜を乾燥させ、富士フィルム社製 X 線フィルム、スーパー HR-H と増感スクリーンとを用いて -80℃ にてオートラジオグラフィーを行った。オートラジオグラム上に 6 個のシグナルが検出されたので、それぞれのシグナルに相当するアガープレート上の位置からトップ・アガロースを切り出し、1% のクロロホルムを添加した 0.5 ml の SM バッファーにそれぞれ浸漬して一夜放置し、ファージを抽出した。それぞれのファージ抽出液を SM バッファーで 1000 倍に希釈し、その中から 1 μ l と 20 μ l を取り、再び上記大腸菌に感染させ、トップ・アガロースとともに上記の方法でアガープレートに蒔いた。ファージをナイロン膜に転写後、上記の方法でプレハイブリダイ

ゼーション、ハイブリダイゼーション、洗浄、乾燥、オートラジオグラフィーを行った。このファージ純化の操作を当初オートラジオグラフィーで検出された6個のシグナル全部について行い、アガープレート上のすべてのファージブランクがcDNAプローブとハイブリダイズするまで繰り返した。純化されたファージのブランクを切り出し、1%クロロホルムを含むSMバッファ0.5mlに浸漬し、4℃で保存した。こうして得られた6種の純化ファージを、それぞれλOIF3, λOIF8, λOIF9, λOIF11, λOIF12, λOIF17と名付けた。

II) 制限酵素消化及びサザンブロット・ハイブリダイゼーションによるヒトOCIFゲノムDNAクローンの分析

純化された6種のファージのDNAを、Molecular Cloning: A Laboratory Manual に書かれた方法に従ってプレートリシス法によって精製した。これらのDNAを制限酵素によって消化し、得られたフラグメントをアガロース電気泳動によって分離した。またアガロース・ゲルで分離されたフラグメントを、一般的な方法でナイロン膜に転移させたのち、OCIFcDNAをプローブとしてサザンブロット・ハイブリダイゼーションを行った。これらの分析の結果、それぞれ純化された6種のファージは異なったクローンであることが判明した。制限酵素消化によって得られたDNAフラグメントのうち、OCIFcDNAとハイブリダイズするものについては、プラスミドベクターにサブクローンした後に下記の方法で塩基配列の分析を行った。

iii) ゲノムDNAクローンから制限酵素消化によって得られたDNAフラグメントのプラスミド・ベクターへのサブクローニングと塩基配列の決定

λOIF8 DNAを制限酵素EcoRI とNotIによって消化し、生じたフラグメントを0.7%アガロースゲルに供与して分離した。5.8kb のEcoRI/NotIフラグメントをQIAEX II Gel Extraction Kit(キアゲン社)を用いて添付されたプロトコールに従ってゲルから抽出した。このフラグメントを、前もってEcoRI とNotIによって切断しておいたpBluescriptII SK+ ベクター(ストラタジーン社)とReady-To-Go T4 Ligase(ファルマシア社)を用いて添付のプロトコールに従ってライゲーションした。得られたリコンビナント・プラスミドを、コンピテントDH5α大腸菌

(アマシャム社)に導入した後、50 μ g/mlのアンピシリンを含有するアガロースプレート上に蒔いてプラスミドを有する大腸菌を選択した。以上のようにして作製された5.8kb EcoRI/NotIフラグメントを有するリコンビナント・プラスミドを、pBSG8-5.8 と命名した。次に、pBSG8-5.8 を制限酵素HindIII で消化して生ずる0.9 kbのDNAフラグメントをアガロースゲルで分離し、上記の方法にしたがって抽出した後、HindIII で前もって切断しておいたpBluescriptII SK-(ストラタジーン社)に挿入して、上記の方法に従ってクローニングした。この0.9kbのHindIII フラグメントを有するリコンビナント・プラスミドを、pBS8H0.9と命名した。一方、 λ OIF11のDNAをEcoRIを用いて消化して生ずる6 kb、3.6kb、及び2.6kbのフラグメントをそれぞれ単離したのち、上記と同様の方法に従ってpBluescriptII SK+ベクターに挿入してクローニングした。こうして作製した6 kb、3.6 kb、及び2.6kb のEcoRI フラグメントを有するリコンビナント・プラスミドを、それぞれpBSG11-6、pBSG11-3.6、pBSG11-2.6と命名した。さらに、pBSG11-6を制限酵素HindIII によって消化することによって生ずる、2.2kb、1.1kb、1.05kbの3種のフラグメントをアガロースゲル電気泳動によって分離し、それぞれpBluescriptII SK-のHindIII サイトに挿入してクローニングした。これら2.2kb、1.1kb、1.05 kb のHindIII フラグメントを有するリコンビナント・プラスミドを、それぞれpBS6H2.2、pBS6H1.1、pBS6H1.05 と命名した。ゲノムDNAの塩基配列の分析には、ABI Dyedexy Terminator Cycle Sequencing Ready Reaction Kit (パーキンエルマー社)と373 DNA Sequencing System (アプライドバイオシステムズ社)を使用した。Molecular Cloning:A Laboratory Manual に書かれた方法に従ってpBSG8-5.8、pBS8H0.9、pBSG11-6、pBSG11-3.6、pBSG11-2.6、pBS6H2.2、pBS6H1.1、pBS6H1.05 を調製し、塩基配列決定用の鋳型として用いた。ヒトO C I FゲノムDNAの塩基配列を配列表配列番号104 及び105 に示す。エクソン1とエクソン2の間に介在する塩基の配列は必ずしも全部は決定されておらず、配列表配列番号104 及び105 に示された塩基配列の間に、およそ17kbのヌクレオチドが介在することが確認されている。

〔実施例 24〕

E I A による O C I F の定量i) ウサギ抗 O C I F 抗体の調製

雄性日本白色ウサギ（体重2.5 ～3.0kg、北山ラベス社より入手）3羽に、r O C I F 200 μ g/mlをフロイント完全アジュバント（DIFCO社）と等量混合してエマルジョンとしたものを、1回1mlずつ皮下免疫した。免疫は1週間隔で合計6回行い、最終免疫後10日目に全採血を行った。分離した血清から抗体を以下の様に精製した。即ち、P B S にて2倍希釈した抗血清に最終濃度40w/v %となるように硫酸アンモニウムを添加して4℃1時間放置後、8000×gで20分間遠心分離を行い、沈殿を得た。沈殿を少量のP B S に溶解し、P B S に対して4℃で透析した後、Protein G-Sepharose カラム（ファルマシア社）に負荷した。P B S にて洗浄後、0.1Mグリシン塩酸緩衝液（pH3.0）にて吸着した免疫グロブリンGを溶出し、直ちに1.5 M トリス塩酸緩衝液（pH8.7）で中性pHとした。溶出蛋白質画分をP B S に対して透析後、280nmにおける吸光度を測定し、その濃度を決定した（E_{1%}^{1cm} 13.5）。西洋ワサビパーオキシダーゼ標識した抗O C I F抗体は、マレイミド活性化パーオキシダーゼキット（ピアス社）を用いて作製した。即ち、1mgの精製抗体に80 μ gのN-スクシンイミド-S-アセチルチオ酢酸を添加し、室温で30分間反応させた。これに5mgのヒドロキシルアミンを添加して脱アセチル化した後、修飾された抗体をボリアクリルアミド脱塩カラムにて分画した。蛋白質画分を1mgのマレイミド活性化パーオキシダーゼと混合し、室温で1時間反応させ酵素標識抗体を得た。

ii) サンドイッチ E I A による O C I F の定量

96ウェルのマイクロタイタープレート（MaxiSorp Immunoplate, Nunc社）の各ウェルに、100 μ lのウサギ抗O C I F抗体（2 μ g/ml、50mM 炭酸緩衝液（pH 9.6））を添加し4℃にて一晩静置して、抗体を固相化した。P B S にて調製した25%ブロッケース（雪印乳業社）を300 μ lずつ各ウェルに添加し、37℃で1時間放置してブロッキングした後、検体（100 μ l/ウェル）を添加し室温で2時間反応させた。0.05% Tween20を含むP B S（PBST）にて3回洗浄した後、10000倍

希釈した西洋ワサビパーオキシダーゼ標識抗OCIF抗体を100 μ lずつ添加し室温で2時間インキュベートした。PBSTにて3回洗浄した後、100 μ lの酵素基質溶液(TMB、ScyTek社)を加え室温で発色させた後、反応を停止した。

450nmにおける吸光度をマイクロプレートリーダー(イムノリーダー NJ2000、日本インターメッド社)を用いて測定し、精製した組み換えOCIFを標準とした検量線から、検体のOCIF濃度を定量した。OCIFの検量線を図13に示す。

[実施例25]

抗OCIFモノクローナル抗体

i) ヒトOCIF抗体産生ハイブリドーマの調製

ヒト線維芽細胞IMR-90を培養し、その培養液から実施例11記載の方法でOCIFを精製した。精製OCIFを10 μ g/100 μ lの濃度になるようにPBSに溶解し、この溶液を2週間おきにBALB/cマウスに腹腔内投与し免疫した。初回及び2回目の免疫においては、等量のフロイド完全アジュバントの混合物を投与した。最終の免疫から3日目に脾臓を摘出し、Bリンパ球を分離し、マウスミエローマ細胞P3x63-AG8.653とを通常用いられているポリエチレングリコール法により細胞融合させた。ついで融合細胞を選択するためにHAT培地で培養を行うことにより、ハイブリドーマ細胞をセレクションした。次に、セレクションされた細胞がOCIF特異的抗体を産生しているか否かを確認するために、0.1M重曹溶液に溶解したOCIF溶液(10 μ g/ml)100 μ lを、96穴マイクロプレート(Nunc社)に加えて作製したソリッドフェーズELISAを用いて、ハイブリドーマ培養液中のOCIF特異的抗体の測定を行った。抗体生産が認められたハイブリドーマを限界希釈法によりクローニングを3-5回繰り返し行い、その都度上記ELISAにより抗体産生量をチェックした。得られた抗体生産株の中から、抗体生産量の高いクローンを選別した。

ii) モノクローナル抗体の生産

実施例25-i)で得た抗体生産株を、それぞれ 1×10^6 を予めプリスタン(アルドリッチケミカル社)を接種しておいたBALB/c系マウスの腹腔内に移植した。移植2週間後、蓄積した腹水を採取し、本発明のモノクローナル抗体を含む

腹水を得た。この腹水より、アフィゲルプロテインAセファロース（バイオラッド社製）を用いたアフィニティクロマトグラフィーにより精製抗体を得た。即ち、腹水を等量のバインディングバッファー（バイオラッド社）で希釈し、プロテインAカラムに負荷した後、充分量の同バッファーで洗浄した。IgGの溶出は、エリューションバッファー（バイオラッド社）で行った。得られた溶出液を水で透析した後、凍結乾燥を行った。得られた精製抗体をSDS-PAGEにより純度検定を行ったところ、分子量約150,000 の位置に均一なバンドを認めた。

iii) OCIFに対して高親和性を有するモノクローナル抗体の選択

実施例25-ii) で得た抗体をPBSに溶解し、ローリー法により蛋白定量を行った。ついで、各抗体を蛋白濃度が一定になるようにPBSに溶解し、この溶液を段階希釈法により希釈した。実施例25-ii) に記載のソリッドフェーズELISAを用いて、高い希釈段階までOCIFと反応するモノクローナル抗体を選別した。その結果、A1G5、E3H8、及びD2F4の3種の抗体が得られた。

iv) 抗体のサブクラスの検定

実施例25-iii)で選択した本発明の抗体のクラス及びサブクラスを、イムノグロブリンクラス及びサブクラス分析キット（アマシャム社）を用いて検定した。検定は、キットに指示されているプロトコールに従って実施した。結果を表15に示す。E3H8、A1G5、及びD2F4は、それぞれIgG₁、IgG_{2a}、及びIgG_{2b}であった。

第15表

抗体名	IgG ₁	IgG _{2a}	IgG _{2b}	IgG ₃	IgA	IgM	κ
A 1 G 5	—	+	—	—	—	—	+
E 3 H 8	+	—	—	—	—	—	+
D 2 F 4	—	—	+	—	—	—	+

v) OCIFのELISAによる測定方法

実施例25-iv) で得たA1G5、E3H8、及びD2F4の3種のモノクローナル抗体を、それぞれ固相抗体と標識抗体とした。それぞれの組み合わせにより、サンドイッチELISAを構築した。抗体の標識は、マレイミド活性化パーオキシダーゼキ

ット（ピアス社）を用いて行った。各々の抗体を $10\mu\text{g/ml}$ の濃度になるように 0.1M 重曹溶液に溶解し、96穴イムノプレート（Nunc 社）の各ウエル当たり $100\mu\text{l}$ ずつそれぞれ分注し、室温で一晩放置した。次いで、各々のプレートを $1/2$ 濃度のブロックエース（雪印乳業社）でブロックし、 0.1% のTween20 を含むPBS（洗浄バッファー）で3回洗浄した。各濃度のOCIFを第一次反応バッファー（ $1/2.5$ 濃度のブロックエース及び 0.1% Tween20 を含む 0.2M トリス塩酸緩衝液、 $\text{pH } 7.4$ ）で調製した。調製した各濃度のOCIF溶液 $100\mu\text{l}$ ずつ各ウエルに加え、 37°C で3時間放置し、次いで洗浄バッファーで3回洗浄した。標識抗体の希釈には、第二次反応バッファー（ $1/4$ 濃度のブロックエース及び 0.1% の Tween 20を含む 0.1M トリス塩酸緩衝液、 $\text{pH } 7.4$ ）を用いた。各標識抗体を第2次反応バッファーで400 倍に希釈し、その各々 $100\mu\text{l}$ ずつを各ウエルにそれぞれ添加した。各々のプレートを 37°C で2時間放置し、次いで3回洗浄した後、基質溶液（ 0.4mg/ml のオルトフェニレンジアミン塩酸、 0.006% 過酸化水素を含む 0.1M クエン酸－リン酸バッファー、 $\text{pH } 4.5$ ） $100\mu\text{l}$ を各ウエルに添加した。 37°C で15分間暗室に放置した後、 6N 硫酸 $50\mu\text{l}$ を各ウエルに添加することにより酵素反応を停止させ、イムノリーダー（NJ2000, 日本インターメッド社）を用いて 492nm の吸光度を測定した。3種の抗体をそれぞれ固相抗体或いは標識抗体としたいずれの組み合わせにおいても良好な測定結果が得られ、3種の抗体はそれぞれOCIFの異なるエピトープを認識することを認めた。代表例として、A1G5を固相抗体としE3H8を標識抗体としたときの検量線を図14に示す。

vi) ヒト血清中のOCIFの測定

健常人5名の血清中のOCIFを実施例25-(v)の図14のELISA系で測定した。即ち、A1G5を実施例25-(v)と同様にイムノプレートに固相化し、各ウエルに第1次反応バッファーを $50\mu\text{l}$ 加え、次いで各ヒト血清 $50\mu\text{l}$ を加えて 37°C で3時間放置した。洗浄バッファーで3回洗浄した後、第2次反応バッファーで400 倍に希釈したE3H8の標識抗体 $100\mu\text{l}$ を各ウエルに加えて、 37°C で2時間放置した。プレートを洗浄バッファーで3回洗浄後、上記基質溶液 $100\mu\text{l}$ を各ウエルに添加し、 37°C で15分間反応させた。各ウエルに 6N 硫酸 $50\mu\text{l}$ ずつ添加し

て酵素反応を停止させ、イムノリーダーで492nmの吸光度を測定した。既知量のOCIFを含む第1次反応バッファーについても同様に操作し、図14に示すようなOCIFの検量線を作成し、血清試料の吸光度から血清中のOCIF量を求めた。結果を表16に示す。

第16表

血清サンプル	OCIF量 (ng/ml)
1	5.0
2	2.0
3	1.0
4	3.0
5	1.5

〔実施例26〕

骨粗鬆症に対する治療効果

神経切除による不動性の骨萎縮モデルに対するOCIFの治療効果を確認した。Fischer系雄ラットを用い、6週齢（体重約120g）で左上腕神経叢を切除することにより、左前肢の不動化を惹起して骨萎縮モデルを作成した。OCIFは0.01%Tween80を含むPBS（－）で調整し、翌日から5 μ g/kg及び50 μ g/kgの用量で12時間間隔で1日2回、2週間連日静脈内投与した。正常群には偽手術を施し、対照群には0.01%Tween80を含むPBS（－）を同様に投与した。投与終了後、左上腕を摘出し骨強度を測定した。結果を図15に示す。

この結果、正常群に比べ対照群では骨強度の低下が観察されたが、OCIF 50 μ g/kg投与群において改善が認められた。

産業上の利用可能性

本発明により、新規な破骨細胞形成抑制活性を有する蛋白質及びその効率的な製造方法が提供される。本発明の蛋白質は破骨細胞形成抑制活性を有し、骨粗鬆症等各種の骨量減少性疾患の治療剤として或いはこれらの疾患の免疫学的診断の

ための抗原等として利用することができる。

寄託された微生物への言及

寄託機関の名称及びあて名

名 称：通商産業省工業技術院生命工学工業技術研究所

あて名：日本国茨城県つくば市東1丁目1番3号（郵便番号305）

寄託機関に寄託した日

平成7年6月21日（原寄託日）

（平成7年6月21日に寄託された微工研菌寄第P-14998号より移管、移管日

平成7年10月25日）

受託番号 F E R M BP-5267

配 列 表

配列番号 : 1

配列の長さ : 6

配列の型 : アミノ酸

トポロジー : 直鎖状

配列の種類 : ペプチド (蛋白質の内部アミノ酸)

配列 :

Xaa Tyr His Phe Pro Lys

1 5

配列番号 : 2

配列の長さ : 14

配列の型 : アミノ酸

トポロジー : 直鎖状

配列の種類 : ペプチド (蛋白質の内部アミノ酸)

配列 :

Xaa Gln His Ser Xaa Gln Glu Gln Thr Phe Gln Leu Xaa Lys

1 5 10

配列番号 : 3

配列の長さ : 12

配列の型 : アミノ酸

トポロジー : 直鎖状

配列の種類 : ペプチド (蛋白質の内部アミノ酸)

配列 :

Xaa Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys

1 5 10

配列番号 : 4

配列の長さ : 380

配列の型 : アミノ酸

トポロジー : 直鎖状

配列の種類 : 蛋白質 (OCIF ; シグナル無し)

配列 :

Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser			
1	5	10	15
His Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys			
	20	25	30
Gln His Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro			
	35	40	45
Asp His Tyr Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu			
	50	55	60
Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu			
	65	70	75
Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Lys Glu Gly Arg			
	80	85	90
Tyr Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro			
	95	100	105
Gly Phe Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val			
	110	115	120
Cys Lys Arg Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser			
	125	130	135
Lys Ala Pro Cys Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu			
	140	145	150
Leu Leu Thr Gln Lys Gly Asn Ala Thr His Asp Asn Ile Cys Ser			
	155	160	165

Gly Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile Asp Val Thr Leu	170	175	180
Cys Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr Lys Phe Thr	185	190	195
Pro Asn Trp Leu Ser Val Leu Val Asp Asn Leu Pro Gly Thr Lys	200	205	210
Val Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser Ser	215	220	225
Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn	230	235	240
Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu	245	250	255
Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr	260	265	270
Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys	275	280	285
Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro	290	295	300
Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn	305	310	315
Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His	320	325	330
Ser Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys	335	340	345
Lys Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr	350	355	360
Gln Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val	365	370	375

Lys Ile Ser Cys Leu

380

配列番号 : 5

配列の長さ : 4 0 1

配列の型 : アミノ酸

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F ; シグナル含む)

配列 :

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

-20

-15

-10

Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His

-5

-1 1

5

Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro

10

15

20

Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr

25

30

35

Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His

40

45

50

Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu

55

60

65

Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys

70

75

80

Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys

85

90

95

His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr

100

105

110

Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320

Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
370 375 380

配列：

ATGAACAAC	TGCTGTGCTG	CGCGCTCGTG	TTTCTGGACA	TCTCCATTAA	GTGGACCACC	60
CAGGAAACGT	TTCCTCCAAA	GTACCTTCAT	TATGACGAAG	AAACCTCTCA	TCAGCTGTTG	120
TGTGACAAAT	GTCCTCCTGG	TACCTACCTA	AAACAACACT	GTACAGCAAA	GTGGAAGACC	180
GTGTGCGCCC	CTTGCCCTGA	CCACTACTAC	ACAGACAGCT	GGCACACCAG	TGACGAGTGT	240
CTATACTGCA	GCCCCGTGTG	CAAGGAGCTG	CAGTACGTCA	AGCAGGAGTG	CAATCGCACC	300
CACAACCGCG	TGTGCGAATG	CAAGGAAGGG	CGCTACCTTG	AGATAGAGTT	CTGCTTGAAA	360
CATAGGAGCT	GCCCTCCTGG	ATTGAGAGTG	GTGCAAGCTG	GAACCCAGAG	GCGAAATACA	420
GTTTGCAAAA	GATGTCCAGA	TGGGTTCTTC	TCAAATGAGA	CGTCATCTAA	AGCACCTGT	480
AGAAAACACA	CAAATTGCAG	TGTCTTTGGT	CTCCTGCTAA	CTCAGAAAGG	AAATGCAACA	540
CACGACAACA	TATGTTCCGG	AAACAGTGAA	TCAACTCAAA	AATGTGGAAT	AGATGTTACC	600
CTGTGTGAGG	AGGCATTCTT	CAGGTTTGCT	GTTCTTACAA	AGTTTACGCC	TAACTGGCTT	660

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AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAC1 1080
GTCCTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
TATCAGAAGT TATTTT1TAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200
TTATAA

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1206

配列番号 : 7

配列の長さ : 1 5

配列の型 : アミノ酸

トポロジー : 直鎖状

配列の種類 : ペプチド (蛋白質のN末端アミノ酸)

配列 :

Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His	Tyr	Asp	Glu	Glu	Thr	Ser
1			5				10						15	

配列番号 : 8

配列の長さ : 1 1 8 5

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF2)

配列 :

ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGTGC AATCGCACCC ACAACCGCGT GTGCGAATGC 300
AAGGAAGGGC GCTACCTTGA GATAGAGTTC TGCTTGAAAC ATAGGAGCTG CCCTCCTGGA 360
TTTGGAGTGG TGCAAGCTGG AACCCAGAG CGAAATACAG TTTGCAAAAG ATGTCCAGAT 420
GGGTTCTTCT CAAATGAGAC GTCATCTAAA GCACCCTGTA GAAAACACAC AAATTGCAGT 480
GTCTTTGGTC TCCTGCTAAC TCAGAAAGGA AATGCAACAC ACGACAACAT ATGTTCCGGA 540
AACAGTGAAT CAACTCAAAA ATGTGGAATA GATGTTACCC TGTGTGAGGA GGCATTCTTC 600
AGGTTTGCTG TTCCTACAAA GTTTACGCCT AACTGGCTTA GTGTCTTGGT AGACAATTTG 660
CCTGGCACCA AAGTAAACGC AGAGAGTGTA GAGAGGATAA AACGGCAACA CAGCTCACAA 720
GAACAGACTT TCCAGCTGCT GAAGTTATGG AAACATCAAA ACAAAGACCA AGATATAGTC 780
AAGAAGATCA TCCAAGATAT TGACCTCTGT GAAAACAGCG TGCAGCGGCA CATTGGACAT 840
GCTAACCTCA CCTTCGAGCA GCTTCGTAGC TTGATGGAAA GCTTACCGGG AAAGAAAGTG 900
GGAGCAGAAG ACATTGAAAA AACAATAAAG GCATGCAAAC CCAGTGACCA GATCCTGAAG 960
CTGCTCAGTT TGTGGCGAAT AAAAAATGGC GACCAAGACA CCTTGAAGGG CCTAATGCAC 1020
GCACTAAAGC ACTCAAAGAC GTACCACTTT CCCAAACTG TCACTCAGAG TCTAAAGAAG 1080
ACCATCAGGT TCCTTCACAG CTTCACAATG TACAAATTGT ATCAGAAGTT ATTTT TAGAA 1140
ATGATAGGTA ACCAGGTCCA ATCAGTAAAA ATAAGCTGCT TATAA 1185

配列番号 : 9

配列の長さ : 3 9 4

配列の型 : アミノ酸

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F 2)

配列 :

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15					-10				
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					1					5				
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Cys
55					60					65				
Asn	Arg	Thr	His	Asn	Arg	Val	Cys	Glu	Cys	Lys	Glu	Gly	Arg	Tyr
70					75					80				
Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys	His	Arg	Ser	Cys	Pro	Pro	Gly
85					90					95				
Phe	Gly	Val	Val	Gln	Ala	Gly	Thr	Pro	Glu	Arg	Asn	Thr	Val	Cys
100					105					110				
Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe	Ser	Asn	Glu	Thr	Ser	Ser	Lys
115					120					125				
Ala	Pro	Cys	Arg	Lys	His	Thr	Asn	Cys	Ser	Val	Phe	Gly	Leu	Leu
130					135					140				
Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr	His	Asp	Asn	Ile	Cys	Ser	Gly
145					150					155				
Asn	Ser	Glu	Ser	Thr	Gln	Lys	Cys	Gly	Ile	Asp	Val	Thr	Leu	Cys
160					165					170				
Glu	Glu	Ala	Phe	Phe	Arg	Phe	Ala	Val	Pro	Thr	Lys	Phe	Thr	Pro
175					180					185				

Asn Trp Leu Ser Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val
 190 195 200
 Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln
 205 210 215
 Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys
 220 225 230
 Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys
 235 240 245
 Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe
 250 255 260
 Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val
 265 270 275
 Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser
 280 285 290
 Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly
 295 300 305
 Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser
 310 315 320
 Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys
 325 330 335
 Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln
 340 345 350
 Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys
 355 360 365
 Ile Ser Cys Leu
 370

配列番号 : 1 0

配列の長さ : 1 0 8 9

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : c DNA (O C I F 3)

配列 :

```
ATGAACAAGT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCTACAA AGTTTACGCC TAACTGGCT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC AGTTTGTGGC GAATAAAAAA TGGCGACCAA 900
GACACCTTGA AGGGCCTAAT GCACGCACTA AAGCACTCAA AGACGTACCA CTTTCCCAAA 960
ACTGTCACTC AGAGTCTAAA GAAGACCATC AGGTTCTTTC ACAGCTTCAC AATGTACAAA 1020
TTGTATCAGA AGTTATTTTT AGAAATGATA GGTAACCAGG TCCAATCAGT AAAAATAAGC 1080
TGCTTATAA 1089
```

配列番号 : 1 1

配列の長さ : 3 6 2

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F 3)

配列 :

Met	Asn	Lys	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15					-10				
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					1					5				
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105					110				
Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
115					120					125				

Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln		
265	270	275
Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr		
280	285	290
Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile		
295	300	305
Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu		
310	315	320
Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser		
325	330	335

Cys Leu

340

配列番号 : 1 2

配列の長さ : 4 6 5

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF4)

配列 :

```
ATGAACAAGT TGCTGTGCTG CTCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTC 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GTACGTGTCA ATGTGCAGCA 420
AAATTAATTA GGATCATGCA AAGTCAGATA GTTGTGACAG TTTAG 465
```

配列番号 : 1 3

配列の長さ : 1 5 4

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (OCIF4)

配列 :

Met Asn Lys Leu Leu Cys Cys Ser Leu Val Phe Leu Asp Ile Ser
 -20 -15 -10
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
 -5 1 5
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
 10 15 20
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
 25 30 35
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
 40 45 50
 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
 55 60 65
 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
 70 75 80
 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
 85 90 95
 His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr
 100 105 110
 Cys Gln Cys Ala Ala Lys Leu Ile Arg Ile Met Gln Ser Gln Ile
 115 120 125
 Val Val Thr Val
 130

配列番号 : 1 4

配列の長さ : 4 3 8

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : c D N A (O C I F 5)

配列 :

```

ATGAACAAGT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GATGCAGGAG AAGACCCAAG 420
CCACAGATAT GTATCTGA 438

```

配列番号 : 1 5

配列の長さ : 1 4 0

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F 5)

配列 :

```

Met Asn Lys Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
-20 -15 -10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
-5 1 5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
10 15 20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
25 30 35

```

Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Cys
100					105					110				
Arg	Arg	Arg	Pro	Lys	Pro	Gln	Ile	Cys	Ile					
115					120					125				

配列番号 : 1 6

配列の長さ : 2 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーT3)

配列 :

AATTAACCCT CACTAAAGGG

20

配列番号 : 1 7

配列の長さ : 2 2

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類：合成DNA（プライマーT7）

配列：

GTAATACGAC TCACTATAGG GC

22

配列番号：18

配列の長さ：20

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーIF1）

配列：

ACATCAAAAC AAAGACCAAG

20

配列番号：19

配列の長さ：20

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーIF2）

配列：

TCTTGGTCTT TGTTTTGATG

20

配列番号：20

配列の長さ：20

配列の型：核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成 DNA (プライマー IF3)

配列 :

TTATTCGCCA CAACTGAGC

20

配列番号 : 2 1

配列の長さ : 2 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成 DNA (プライマー IF4)

配列 :

TTGTGAAGCT GTGAAGGAAC

20

配列番号 : 2 2

配列の長さ : 2 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成 DNA (プライマー IF5)

配列 :

GCTCAGTTTG TGGCGAATAA

20

配列番号 : 2 3

配列の長さ : 2 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーIF6)

配列 :

GTGGGAGCAG AAGACATTGA

20

配列番号 : 2 4

配列の長さ : 2 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーIF7)

配列 :

AATGAACAAC TTGCTGTGCT

20

配列番号 : 2 5

配列の長さ : 2 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーIF8)

配列 :

TGACAAATGT CCTCCTGGTA

20

配列番号 : 2 6

配列の長さ : 2 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーIF9)

配列 :

AGGTAGGTAC CAGGAGGACA

20

配列番号 : 2 7

配列の長さ : 2 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーIF10)

配列 :

GAGCTGCCCT CCTGGATTG

20

配列番号 : 2 8

配列の長さ : 2 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーIF11)

配列：

CAAACTGTAT TTCGCTCTGG

20

配列番号：29

配列の長さ：20

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーIF12）

配列：

GTGTGAGGAG GCATTCTTCA

20

配列番号：30

配列の長さ：32

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーC19SF）

配列：

GAATCAACTC AAAAAAGTGG AATAGATGTT AC

32

配列番号：31

配列の長さ：32

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーC19SR）

配列：

GTAACATCTA TTCCACTTTT TTGAGTTGAT TC

32

配列番号：32

配列の長さ：30

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーC20SF）

配列：

ATAGATGTTA CCCTGAGTGA GGAGGCATTC

30

配列番号：33

配列の長さ：30

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーC20SR）

配列：

GAATGCCTCC TCACTCAGGG TAACATCTAT

30

配列番号 : 3 4

配列の長さ : 3 1

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーC21SF)

配列 :

CAAGATATTG ACCTCAGTGA AAACAGCGTG C

31

配列番号 : 3 5

配列の長さ : 3 1

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーC21SR)

配列 :

GCACGCTGTT TTCACTGAGG TCAATATCTT G

31

配列番号 : 3 6

配列の長さ : 3 1

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーC22SF)

配列 :

AAAACAATAA AGGCAAGCAA ACCCAGTGAC C

31

配列番号 : 3 7

配列の長さ : 3 1

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーC22SR)

配列 :

GGTCACTGGG TTTGCTTGCC TTTATTGTTT T

31

配列番号 : 3 8

配列の長さ : 3 1

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーC23SF)

配列 :

TCAGTAAAAA TAAGCAGCTT ATAAC TGGCC A

31

配列番号 : 3 9

配列の長さ : 3 1

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーC23SR)

配列：

TGGCCAGTTA TAAGCTGCTT ATTTTACTG A

31

配列番号：4 0

配列の長さ：2 2

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーIF 14）

配列：

TTGGGGTTTA TTGGAGGAGA TG

22

配列番号：4 1

配列の長さ：3 6

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーDCR1F）

配列：

ACCACCCAGG AACCTTGCCC TGACCACTAC TACACA

36

配列番号：4 2

配列の長さ：3 6

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーDCR1R）

配列：

GTCAGGGCAA GGTTCCTGGG TGGTCCACTT AATGGA

36

配列番号：43

配列の長さ：36

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーDCR2F）

配列：

ACCGTGTGCG CCGAATGCAA GGAAGGGCGC TACCTT

36

配列番号：44

配列の長さ：36

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーDCR2R）

配列：

TTCCTTGCAT TCGGCGCACA CGGTCTTCCA CTTTGC

36

配列番号：45

配列の長さ：36

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーDCR3F）

配列：

AACCGCGTGT GCAGATGTCC AGATGGGTTC TTCTCA

36

配列番号：46

配列の長さ：36

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーDCR3R）

配列：

ATCTGGACAT CTGCACACGC GGTGTGGGT GCGATT

36

配列番号：47

配列の長さ：36

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーDCR4F）

配列：

ACAGTTTGCA AATCCGAAA CAGTGAATCA ACTCAA

36

配列番号 : 4 8

配列の長さ : 3 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成 DNA (プライマー DCR4R)

配列 :

ACTGTTTCCG GATTTGCAAA CTGTATTTCG CTCTGG

36

配列番号 : 4 9

配列の長さ : 3 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成 DNA (プライマー DDD1F)

配列 :

AATGTGGAAT AGATATTGAC CTCTGTGAAA ACAGCG

36

配列番号 : 5 0

配列の長さ : 3 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成 DNA (プライマー DDD1R)

配列 :

AGAGGTCAAT ATCTATTCCA CATTTTGTGAG TTGATT

36

配列番号 : 5 1

配列の長さ : 3 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーDDD2F)

配列 :

AGATCATCCA AGACGCACTA AAGCACTCAA AGACGT

36

配列番号 : 5 2

配列の長さ : 3 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーDDD2R)

配列 :

GCTTTAGTGC GTCTTGGATG ATCTTCTTGA CTATAT

36

配列番号 : 5 3

配列の長さ : 2 9

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 合成DNA (プライマーXhoI F)

配列：

GGCTCGAGCG CCCAGCCGCC GCCTCCAAG

29

配列番号：5 4

配列の長さ：2 0

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーIF 16）

配列：

TTTGAGTGCT TTAGTGCGTG

20

配列番号：5 5

配列の長さ：3 0

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーCL F）

配列：

TCAGTAAAAA TAAGCTAACT GGAAATGGCC

30

配列番号：5 6

配列の長さ：3 0

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーCL R）

配列：

GGCCATTTCC AGTTAGCTTA TTTTACTGA

30

配列番号：57

配列の長さ：29

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーCC R）

配列：

CCGGATCCTC AGTGCTTTAG TCGTGTCAT

29

配列番号：58

配列の長さ：29

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーCCD2 R）

配列：

CCGGATCCTC ATTGGATGAT CTTCTTGAC

29

配列番号：59

配列の長さ：29

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーCCD1 R）

配列：

CCGGATCCTC ATATTCCACA TTTTGTAGT

29

配列番号：60

配列の長さ：29

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーCCR4 R）

配列：

CCGGATCCTC ATTTGCAAAC TGTATTTCG

29

配列番号：61

配列の長さ：29

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：合成DNA（プライマーCCR3 R）

配列：

CCGGATCCTC ATTCGCACAC GCGGTTGTG

29

配列番号 : 6 2

配列の長さ : 4 0 1

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C I 9 S)

配列 :

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15						-10			
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1					5			
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15						20			
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30						35			
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45						50			
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60						65			
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75						80			
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90						95			
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105						110			
Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
115					120						125			

Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Ser		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335

Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe
 340 345 350
 Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly
 355 360 365
 Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
 370 375 380

配列番号 : 6 3

配列の長さ : 4 0 1

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C 2 0 S)

配列 :

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
 -20 -15 -10
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
 -5 -1 1 5
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
 10 15 20
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
 25 30 35
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
 40 45 50
 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
 55 60 65
 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
 70 75 80

Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
85	90	95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Ser Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290


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Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser
295              300              305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu
310              315              320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr
325              330              335
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe
340              345              350
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly
355              360              365
Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
370              375              380

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配列番号 : 6 4

配列の長さ : 4 0 1

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C 2 1 S)

配列 :

```

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
-20              -15              -10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
-5              -1   1              5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
10              15              20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
25              30              35

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Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
40	45	50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
55	60	65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
70	75	80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
85	90	95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245

Ile Gln Asp Ile Asp Leu Ser Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
340	345	350
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
355	360	365
Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
370	375	380

配列番号 : 6 5

配列の長さ : 4 0 1

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C 2 2 S)

配列 :

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

-20

-15

-10

Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25	30	35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
40	45	50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
55	60	65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
70	75	80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
85	90	95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200

Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
205 210 215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys
220 225 230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile
235 240 245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile
250 255 260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu
265 270 275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr
280 285 290
Ile Lys Ala Ser Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser
295 300 305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu
310 315 320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr
325 330 335
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe
340 345 350
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly
355 360 365
Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
370 375 380

配列番号 : 6 6

配列の長さ : 4 0 1

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C 2 3 S)

配列 :

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15					-10				
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1				5				
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105					110				
Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
115					120					125				
Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
130					135					140				
Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr
145					150					155				

His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
340	345	350
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
355	360	365

Asn Gln Val Gln Ser Val Lys Ile Ser Ser Leu
 370 375 380

配列番号 : 6 7

配列の長さ : 3 6 0

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - D C R 1)

配列 :

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
 -20 -15 -10
 Ile Lys Trp Thr Thr Gln Glu Pro Cys Pro Asp His Tyr Tyr Thr
 -5 -1 1 5
 Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val
 10 15 20
 Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
 25 30 35
 Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu
 40 45 50
 Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val
 55 60 65
 Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro
 70 75 80
 Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg
 85 90 95
 Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys
 100 105 110

Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser		
115	120	125
Thr Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe		
130	135	140
Phe Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser		
145	150	155
Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser		
160	165	170
Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe		
175	180	185
Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile		
190	195	200
Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val		
205	210	215
Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg		
220	225	230
Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp		
235	240	245
Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu		
250	255	260
Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr		
265	270	275
Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His		
280	285	290
Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe		
295	300	305
Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu		
310	315	320

Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
 325 330 335

配列番号 : 6 8

配列の長さ : 3 5 9

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - D C R 2)

配列 :

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
 -20 -15 -10
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
 -5 -1 1 5
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
 10 15 20
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
 25 30 35
 Val Cys Ala Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
 40 45 50
 Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln
 55 60 65
 Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp
 70 75 80
 Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys
 85 90 95
 His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly
 100 105 110

Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr		
115	120	125
Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe		
130	135	140
Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val		
145	150	155
Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val		
160	165	170
Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln		
175	180	185
Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val		
190	195	200
Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln		
205	210	215
Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser		
220	225	230
Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile		
235	240	245
Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys		
250	255	260
Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu		
265	270	275
Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe		
280	285	290
Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu		
295	300	305
His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu		
310	315	320

Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu

325

330

335

配列番号 : 6 9

配列の長さ : 3 6 3

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - D C R 3)

配列 :

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15							-10		
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1					5			
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Arg	Cys	Pro	Asp	Gly	Phe	Phe	Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala
85					90					95				
Pro	Cys	Arg	Lys	His	Thr	Asn	Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu
100					105					110				

Thr Gln Lys Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn
 115 120 125
 Ser Glu Ser Thr Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu
 130 135 140
 Glu Ala Phe Phe Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn
 145 150 155
 Trp Leu Ser Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn
 160 165 170
 Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu
 175 180 185
 Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp
 190 195 200
 Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu
 205 210 215
 Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu
 220 225 230
 Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly
 235 240 245
 Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp
 250 255 260
 Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp
 265 270 275
 Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys
 280 285 290
 Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr
 295 300 305
 Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys
 310 315 320

Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile

325

330

335

Ser Cys Leu

340

配列番号 : 7 0

配列の長さ : 3 5 9

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - D C R 4)

配列 :

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

-20

-15

-10

Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His

-5

-1 1

5

Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro

10

15

20

Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr

25

30

35

Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His

40

45

50

Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu

55

60

65

Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys

70

75

80

Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys

85

90

95

His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr
 100 105 110
 Pro Glu Arg Asn Thr Val Cys Lys Ser Gly Asn Ser Glu Ser Thr
 115 120 125
 Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe
 130 135 140
 Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val
 145 150 155
 Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val
 160 165 170
 Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln
 175 180 185
 Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val
 190 195 200
 Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln
 205 210 215
 Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser
 220 225 230
 Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile
 235 240 245
 Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys
 250 255 260
 Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu
 265 270 275
 Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe
 280 285 290
 Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu
 295 300 305

His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu
 310 315 320
 Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
 325 330 335

配列番号 : 7 1

配列の長さ : 3 2 6

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - D D D 1)

配列 :

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
 -20 -15 -10
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
 -5 -1 1 5
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
 10 15 20
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
 25 30 35
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
 40 45 50
 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
 55 60 65
 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
 70 75 80
 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
 85 90 95

His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
175	180	185
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
190	195	200
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
205	210	215
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
220	225	230
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
235	240	245
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
250	255	260
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
265	270	275
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
280	285	290
Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
295	300	305

配列番号 : 7 2

配列の長さ : 3 2 7

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - D D D 2)

配列 :

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15						-10			
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1					5			
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105					110				
Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
115					120					125				

Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys		
250	255	260
Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser		
265	270	275
Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile		
280	285	290
Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
295	300	305

配列番号 : 7 3

配列の長さ : 3 9 9

配列の型 : アミノ酸

鎖の数：1

トポロジー：直鎖状

配列の種類：蛋白質 (O C I F - C L)

配列：

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15						-10			
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1					5			
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105					110				
Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
115					120					125				
Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
130					135					140				
Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr
145					150					155				

His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
340	345	350
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
355	360	365

Asn Gln Val Gln Ser Val Lys Ile Ser

370

375

配列番号 : 7 4

配列の長さ : 3 5 1

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C C)

配列 :

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15					-10				
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1				5				
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105					110				

Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320

Met His Ala Leu Lys His

325

330

配列番号 : 7 5

配列の長さ : 2 7 2

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C D D 2)

配列 :

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15					-10				
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1				5				
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105					110				

Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe
115 120 125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130 135 140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr
145 150 155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys
160 165 170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala
175 180 185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp
190 195 200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
205 210 215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys
220 225 230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile
235 240 245
Ile Gln
250

配列番号 : 7 6

配列の長さ : 1 9 7

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C D D 1)

配列 :

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15						-10			
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1					5			
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15						20			
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30						35			
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45						50			
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60						65			
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75						80			
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90						95			
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105						110			
Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
115					120						125			
Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
130					135						140			
Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr
145					150						155			
His	Asp	Asn	Ile	Cys	Ser	Gly	Asn	Ser	Glu	Ser	Thr	Gln	Lys	Cys
160					165						170			
Gly	Ile													
175														

配列番号 : 7 7

配列の長さ : 1 4 3

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C C R 4)

配列 :

```

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
-20                      -15                      -10

Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
-5                      -1 1                      5

Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
10                      15                      20

Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
25                      30                      35

Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
40                      45                      50

Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
55                      60                      65

Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
70                      75                      80

Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
85                      90                      95

His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr
100                     105                     110

Pro Glu Arg Asn Thr Val Cys Lys
115                     120

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配列番号 : 7 8

配列の長さ : 1 0 6

配列の型 : アミノ酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C C R 3)

配列 :

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

-20

-15

-10

Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His

-5

-1 1

5

Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro

10

15

20

Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr

25

30

35

Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His

40

45

50

Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu

55

60

65

Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys

70

75

80

Glu

85

配列番号：79

配列の長さ：393

配列の型：アミノ酸

トポロジー：直鎖状

配列の種類：蛋白質 (O C I F - C B s t)

配列：

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15						-10			
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1					5			
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105					110				
Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
115					120					125				
Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
130					135					140				

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
340	345	350

Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly

355

360

365

Asn Leu Val

370

配列番号 : 8 0

配列の長さ : 3 2 1

配列の型 : アミノ酸

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C S p h)

配列 :

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

-20

-15

-10

Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His

-5

-1 1

5

Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro

10

15

20

Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr

25

30

35

Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His

40

45

50

Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu

55

60

65

Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys

70

75

80

Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys

85

90

95

His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Ser Leu Asp		
295	300	

配列番号 : 8 1

配列の長さ : 2 0 2

配列の型 : アミノ酸

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C B s p)

配列 :

```

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
-20                      -15                      -10

Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
-5                      -1 1                      5

10                      15                      20

Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
25                      30                      35

Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
40                      45                      50

Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
55                      60                      65

Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
70                      75                      80

Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
85                      90                      95

Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
100                     105                     110

His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr
115                     120                     125

Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe
130                     135                     140

```

Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
 145 150 155
 Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr
 160 165 170
 His Asp Asn Ile Cys Ser Gly
 175 180

配列番号 : 8 2

配列の長さ : 8 4

配列の型 : アミノ酸

トポロジー : 直鎖状

配列の種類 : 蛋白質 (O C I F - C P s t)

配列 :

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
 -20 -15 -10
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
 -5 -1 1 5
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
 10 15 20
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
 25 30 35
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
 40 45 50
 Thr Ser Asp Glu Cys Leu Tyr Leu Val
 55 60

配列番号 : 8 3

配列の長さ : 1 2 0 6

配列の型：核酸

鎖の数：1

トポロジー：直鎖状

配列の種類：cDNA (OCIF-C19S)

配列：

```
ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AAAGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080
GTCACCTCAG GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
TATCAGAAGT TATTTT TAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200
TTATAA 1206
```

配列番号 : 8 4

配列の長さ : 1 2 0 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : c D N A (O C I F - C 2 0 S)

配列 :

```
ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
CTGAGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAACT 1080
GTCCTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
TATCAGAAGT TATTTTTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200
```

TTATAA

1206

配列番号 : 8 5

配列の長さ : 1 2 0 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-C21S)

配列 :

ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCGAGA GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCCT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCAG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080

GTCAC TCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
TATCAGAAGT TATTTT TAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200
TTATAA 1206

配列番号 : 8 6

配列の長さ : 1 2 0 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF - C22S)

配列 :

ATGAACA ACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCA GA GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCAAGCAAA 960

CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080
GTCACCTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
TATCAGAAGT TATTTT TAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200
TTATAA 1206

配列番号 : 8 7

配列の長さ : 1 2 0 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-C23S)

配列 :

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840

GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 90C
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
CCCAGTGACC AGATCCTGAA GCTGCTCACT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080
GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
TATCAGAAGT TATTTT TAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCAGC 1200
TTATAA 1206

配列番号 : 8 8

配列の長さ : 1 0 8 3

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-DCR1)

配列 :

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAACCTT GCCCTGACCA CTACTACACA GACAGCTGGC ACACCAGTGA CGAGTGTCTA 120
TACTGCAGCC CCGTGTGCAA GGAGCTGCAG TACGTCAAGC AGGAGTGCAA TCGCACCCAC 180
AACCGCGTGT GCGAATGCAA GGAAGGGCGC TACCTTGAGA TAGAGTTCTG CTTGAAACAT 240
AGGAGCTGCC CTCCTGGATT TGGAGTGGTG CAAGCTGGAA CCCAGAGCG AAATACAGTT 300
TGCAAAAGAT GTCCAGATGG GTTCTTCTCA AATGAGACGT CATCTAAAGC ACCCTGTAGA 360
AAACACACAA ATTGCAGTGT CTTTGGTCTC CTGCTAACTC AGAAAGGAAA TGCAACACAC 420
GACAACATAT GTTCCGAAA CAGTGAATCA ACTCAAAAAT GTGGAATAGA TGTTACCCTG 480
TGTGAGGAGG CATTCTTCAG GTTTGCTGTT CCTACAAAGT TTACGCCTAA CTGGCTTAGT 540
GTCTTGGTAG ACAATTTGCC TGGCACCAAA GTAAACGCAG AGAGTGTAGA GAGGATAAAA 600
CGGCAACACA GCTCACAAGA ACAGACTTTC CAGCTGCTGA AGTTATGGAA ACATCAAAAC 660
AAAGACCAAG ATATAGTCAA GAAGATCATC CAAGATATTG ACCTCTGTGA AAACAGCGTG 720

CAGCGGCACA TTGGACATGC TAACCTCACC TTCGAGCAGC TTCGTAGCTT GATGGAAAGC 780
TTACCGGGAA AGAAAGTGGG AGCAGAAGAC ATTGAAAAAA CAATAAAGGC ATGCAAACCC 840
AGTGACCAGA TCCTGAAGCT GCTCAGTTTG TGGCGAATAA AAAATGGCGA CCAAGACACC 900
TTGAAGGGCC TAATGCACGC ACTAAAGCAG TCAAAGACGT ACCACTTTCC CAAAACGTGC 960
ACTCAGAGTC TAAAGAAGAC CATCAGGTTC CTTACAGCT TCACAATGTA CAAATTGTAT 1020
CAGAAGTTAT TTTTAGAAAT GATAGGTAAC CAGGTCCAAT CAGTAAAAAT AAGCTGCTTA 1080
TAA 1083

配列番号 : 8 9

配列の長さ : 1 0 8 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : c D N A (O C I F - D C R 2)

配列 :

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCG AATGCAAGGA AGGGCGCTAC CTTGAGATAG AGTTCTGCTT GAAACATAGG 240
AGCTGCCCTC CTGGATTTGG AGTGGTGCAA GCTGGAACCC CAGAGCGAAA TACAGTTTGC 300
AAAAGATGTC CAGATGGGTT CTTCTCAAAT GAGACGTCAT CTAAAGCACC CTGTAGAAAA 360
CACACAAATT GCAGTGTCTT TGGTCTCCTG CTAATCAGA AAGGAAATGC AACACACGAC 420
AACATATGTT CCGGAAACAG TGAATCAACT CAAAAATGTG GAATAGATGT TACCCTGTGT 480
GAGGAGGCAT TCTTCAGGTT TGCTGTTTCT ACAAAGTTTA CGCCTAACTG GCTTAGTGTC 540
TTGGTAGACA ATTTGCCTGG CACCAAAGTA AACGCAGAGA GTGTAGAGAG GATAAAACGG 600
CAACACAGCT CACAAGAACA GACTTTCCAG CTGCTGAAGT TATGGAAACA TCAAAACAAA 660
GACCAAGATA TAGTCAAGAA GATCATCCAA GATATTGACC TCTGTGAAAA CAGCGTGCAG 720

CGGCACATIG GACATGCTAA CCTCACCTTC GAGCAGCTTC GTAGCTTGAT GGAAAGCTTA 780
CCGGGAAAGA AAGTGGGAGC AGAAGACATT GAAAAACAA TAAAGGCATG CAAACCCAGT 840
GACCAGATCC TGAAGCTGCT CAGTTTGTGG CGAATAAAAA ATGGCGACCA AGACACCTTG 900
AAGGGCCTAA TGCACGCACT AAAGCACTCA AAGACGTACC ACTTTCCCAA AACTGTCACT 960
CAGAGTCTAA AGAAGACCAT CAGGTTCTTT CACAGCTTCA CAATGTACAA ATTGTATCAG 1020
AAGTTATTTT TAGAAATGAT AGGTAACCAG GTCCAATCAG TAAAAATAAG CTGCTTATAA 1080

配列番号 : 9 0

配列の長さ : 1 0 9 2

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-DCR3)

配列 :

ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTCT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCAGATG TCCAGATGGG TTCTTCTCAA ATGAGACGTC ATCTAAAGCA 360
CCCTGTAGAA AACACACAAA TTGCAGTGTG TTTGGTCTCC TGCTAACTCA GAAAGGAAAT 420
GCAACACACG ACAACATATG TTCCGGAAAC AGTGAATCAA CTCAAAAATG TGGAATAGAT 480
GTTACCCTGT GTGAGGAGGC ATTCTTCAGG TTTGCTGTTC CTACAAAGTT TACGCCTAAC 540
TGGCTTAGTG TCTTGGTAGA CAATTTGCCT GGCACCAAAG TAAACGCAGA GAGTGTAGAG 600
AGGATAAAAC GGCAACACAG CTCACAAGAA CAGACTTTCC AGCTGCTGAA GTTATGGAAA 660
CATCAAAACA AAGACCAAGA TATAGTCAAG AAGATCATCC AAGATATTGA CCTCTGTGAA 720
AACAGCGTGC AGCGGCACAT TGGACATGCT AACCTCACCT TCGAGCAGCT TCGTAGCTTG 780

ATGGAAAGCT TACCGGGAAA GAAAGTGGGA GCAGAAGACA TTGAAAAAAC AATAAAGGCA 840
TGCAAACCCA GTGACCAGAT CCTGAAGCTG CTCAGTTTGT GGCGAATAAA AAATGGCGAC 900
CAAGACACCT TGAAGGGCCT AATGCACGCA CTAAAGCACT CAAAGACGTA CCACTTTCCC 960
AAACTGTCA CTCAGAGTCT AAAGAAGACC ATCAGGTTCC TTCACAGCTT CACAATGTAC 1020
AAATTGTATC AGAAGTTATT TTTAGAAATG ATAGGTAACC AGGTCCAATC AGTAAAAATA 1080
AGCTGCTTAT AA 1092

配列番号 : 9 1

配列の長さ : 1 0 8 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-DCR4)

配列 :

ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGAG GCGAAATACA 420
GTTTGCAAAT CCGGAAACAG TGAATCAACT CAAAAATGTG GAATAGATGT TACCCTGTGT 480
GAGGAGGCAT TCTTCAGGTT TGCTGTTTCT ACAAAGTTTA CGCCTAACTG GCTTAGTGTC 540
TTGGTAGACA ATTTGCCTGG CACCAAAGTA AACGCAGAGA GTGTAGACAG GATAAACCGG 600
CAACACAGCT CACAAGAACA GACTTTCCAG CTGCTGAAGT TATGGAAACA TCAAAACAAA 660
GACCAAGATA TAGTCAAGAA GATCATCCAA GATATTGACC TCTGTGAAAA CAGCGTGCAG 720
CGGCACATTG GACATGCTAA CCTCACCTTC GAGCAGCTTC GTAGCTTGAT GGAAAGCTTA 780

CCGGGAAAGA AAGTGGGAGC AGAAGACATT GAAAAACAA TAAAGGCATG CAAACCCAGT 840
GACCAGATCC TGAAGCTGCT CAGTTTGTGG CGAATAAAAA ATGGCGACCA AGACACCTTG 900
AAGGGCCTAA TGCACGCACT AAAGCACTCA AAGACGTACC ACTTTCCCAA AACTGTCACT 960
CAGAGTCTAA AGAAGACCAT CAGGTTTCCTT CACAGCTTCA CAATGTACAA ATTGTATCAG 1020
AAGTTATTTT TAGAAATGAT AGGTAACCAG GTCCAATCAG TAAAAATAAG CTGCTTATAA 1080

配列番号 : 9 2

配列の長さ : 9 8 1

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-DDD1)

配列 :

ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATATTGAC 600
CTCTGTGAAA ACAGCGTGCA GCGGCACATT GGACATGCTA ACCTCACCTT CGAGCAGCTT 660
CGTAGCTTGA TGGAAAGCTT ACCGGGAAAG AAAGTGGGAG CAGAAGACAT TGAAAAACAA 720
ATAAAGGCAT GCAAACCCAG TGACCAGATC CTGAAGCTGC TCAGTTTGTG GCGAATAAAA 780
AATGGCGACC AAGACACCTT GAAGGGCCTA ATGCACGCAC TAAAGCACTC AAAGACGTAC 840

CACTTTCCCA AACTGTGAC TCAGAGTCTA AAGAAGACCA TCAGGTTCTT TCACAGCTTC 900
ACAATGTACA AATTGTATCA GAAGTTATTT TTAGAAATGA TAGGTAACCA GGTCCAATCA 960
GTAAAAATAA GCTGCTTATA A 981

配列番号 : 9 3

配列の長さ : 9 8 4

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-DDD2)

配列 :

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGACG CACTAAAGCA CTCAAAGACG 840
TACCACTTTC CCAAACTGT CACTCAGAGT CTAAAGAAGA CCATCAGGTT CCTTCACAGC 900
TTCACAATGT ACAAATTGTA TCAGAAGTTA TTTTGTAGAA TGATAGGTAA CCAGGTCCAA 960

TCAGTAAAAA TAAGCTGCTT ATAA

984

配列番号 : 9 4

配列の長さ : 1 2 0 0

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-CL)

配列 :

ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAATGG CGACCAAGAC 1020
ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAACT 1080

GTCCTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
TATCAGAAGT TATTTT TAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTAA 1200

配列番号 : 9 5

配列の長さ : 1 0 5 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-CC)

配列 :

ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020

GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
TATCAGAAGT TATTTT TAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTAA 1200

配列番号 : 9 5

配列の長さ : 1 0 5 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-CC)

配列 :

ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTCTTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020

ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTGA

105C

配列番号 : 9 6

配列の長さ : 8 1 9

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-CDD2)

配列 :

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAG 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGTG 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAATGA 819

配列番号 : 9 7

配列の長さ : 5 9 4

配列の型 : 核酸

ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTGA

105C

配列番号 : 9 6

配列の長さ : 8 1 9

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-CDD2)

配列 :

ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAATGA 819

配列番号 : 9 7

配列の長さ : 5 9 4

配列の型 : 核酸

CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGAG GCGAAATACA 420
GTTTGCAAAT GA 432

配列番号 : 9 9

配列の長さ : 3 2 1

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-CCR3)

配列 :

ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG A 321

配列番号 : 1 0 0

配列の長さ : 1 1 8 2

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-CBst)

配列 :

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCGAGA GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCCTT TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080
GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
TATCAGAAGT TATTTTGTAGA AATGATAGGT AACCTAGTCT AG 1182

配列番号 : 1 0 1

配列の長さ : 9 6 6

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-CSph)

配列 :

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCTAGTCTA 960
GACTAG 966

配列番号 : 1 0 2

配列の長さ : 5 6 4

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-CBsp)

配列 :

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60

CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG CTAG 564

配列番号 : 1 0 3

配列の長さ : 2 5 5

配列の型 : 核酸

鎖の数 : 1

トポロジー : 直鎖状

配列の種類 : cDNA (OCIF-CPst)

配列 :

ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACCTAG TCTAG 255

配列番号 : 1 0 4

配列の長さ : 1 3 1 7

配列の型 : 核酸

鎖の数 : 2

トポロジー：直鎖状

配列の種類：g e n o m i c DNA (ヒトOCIFゲノムDNA-1)

配列：

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CTGGAGACAT ATAAC TTGAA CACTTGGCCC TGATGGGGAA GCAGCTCTGC AGGGACTTTT 60
TCAGCCATCT GTAAACAATT TCAGTGGCAA CCCGCGAACT GTAATCCATG AATGGGACCA 120
CACTTTACAA GTCATCAAGT CTAAC TTCTA GACCAGGGAA TTAATGGGGG AGACAGCGAA 180
CCCTAGAGCA AAGTGCCAAA CTTCTGTCGA TAGCTTGAGG CTAGTGGAAA GACCTCGAGG 240
AGGCTACTCC AGAAGTTCAG CGCGTAGGAA GCTCCGATAC CAATAGCCCT TTGATGATGG 300
TGGGGTTGGT GAAGGGAACA GTGCTCCGCA AGGTTATCCC TGCCCCAGGC AGTCCAATTT 360
TCACTCTGCA GATTCTCTCT GGCTCTAACT ACCCAGATA ACAAGGAGTG AATGCAGAAT 420
AGCACGGGCT TTAGGGCCAA TCAGACATTA GTTAGAAAAA TTCCTACTAC ATGGTTTATG 480
TAAAC TTGAA GATGAATGAT TGCGAACTCC CCGAAAAGGG CTCAGACAAT GCCATGCATA 540
AAGAGGGGCC CTGTAATTTG AGGTTTCAGA ACCCGAAGTG AAGGGGTCAG GCAGCCGGGT 600
ACGGCGGAAA CTCACAGCTT TCGCCCAGCG AGAGGACAAA GGTCTGGGAC AACTCCAAC 660
TGCGTCCGGA TCTTGGCTGG ATCGGACTCT CAGGGTGGAG GAGACACAAG CACAGCAGCT 720
GCCCAGCGTG TGCCAGCCC TCCCACCGCT GGTCCCGGCT GCCAGGAGGC TGGCCGCTGG 780
CGGGAAGGGG CCGGGAACC TCAGAGCCCC GCGGAGACAG CAGCCGCCTT GTTCCTCAGC 840
CCGGTGGCTT TTTTTTCCCC TGCTCTCCA GGGGACAGAC ACCACCGCCC CACCCCTCAC 900
GCCCCACCTC CCTGGGGGAT CCTTTCGCG CCAGCCCTGA AAGCGTTAAT CCTGGAGCTT 960
TCTGCACACC CCCCGACCGC TCCCGCCCAA GCTTCCTAAA AAAGAAAGGT GCAAAGTTTG 1020
GTCCAGGATA GAAAAATGAC TGATCAAAGG CAGGCGATAC TTCCTGTTGC CGGGACGCTA 1080
TATATAACGT GATGAGCGCA CGGGCTGCGG AGACGCACCG GAGCGCTCGC CCAGCCGCCG 1140
CCTCCAAGCC CCTGAGGTTT CCGGGGACCA CA ATG AAC AAG TTG CTG TGC TGC 1193

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Met Asn Lys Leu Leu Cys Cys

-20

-15

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GCG CTC GTG GTAAGTCCCT GGGCCAGCCG ACGGGTGCCC GGCGCCTGGG 1242

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Ala Leu Val

GAGGCTGCTG CCACCTGGTC TCCCAACCTC CCAGCGGACC GGCGGGGAAA AAGGCTCCAC 1302
 TCGCTCCCTC CCAAG 1317

配列番号 : 1 0 5

配列の長さ :

配列の型 : 核酸

鎖の数 : 2

トポロジー : 直鎖状

配列の種類 : g e n o m i c DNA (ヒトOCIFゲノムDNA - 2)

配列 :

GCTTACTTTG TGCCAAATCT CATTAGGCTT AAGGTAATAC AGGACTTTGA GTCAAATGAT 60
 ACTGTTGCAC ATAAGAACAA ACCTATTTTC ATGCTAAGAT GATGCCACTG TGTTCCTTTC 120
 TCCTTCTAG TTT CTG GAC ATC TCC ATT AAG TGG ACC ACC CAG GAA ACG TTT 171

Phe Leu Asp Ile Ser Ile Lys Trp Thr Thr Gln Glu Thr Phe

-10

-5

-1 +1

CCT CCA AAG TAC CTT CAT TAT GAC GAA GAA ACC TCT CAT CAG CTG TTG 219
 Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His Gln Leu Leu

5

10

15

TGT GAC AAA TGT CCT CCT GGT ACC TAC CTA AAA CAA CAC TGT ACA GCA 267
 Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala
 20 25 30 35

AAG TGG AAG ACC GTG TGC GCC CCT TGC CCT GAC CAC TAC TAC ACA GAC	315
Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp	
40 45 50	
AGC TGG CAC ACC AGT GAC GAG TGT CTA TAC TGC AGC CCC GTG TGC AAG	363
Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys	
55 60 65	
GAG CTG CAG TAC GTC AAG CAG GAG TGC AAT CGC ACC CAC AAC CGC GTG	411
Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val	
70 75 80	
TGC GAA TGC AAG GAA GGG CGC TAC CTT GAG ATA GAG TTC TGC TTG AAA	459
Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys	
85 90 95	
CAT AGG AGC TGC CCT CCT GGA TTT GGA GTG GTG CAA GCT G GTACGTGTCA	509
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala	
100 105 110	
ATGTGCAGCA AAATTAATTA GGATCATGCA AAGTCAGATA GTTGTGACAG TTTAGGAGAA	569
CACTTTTGTT CTGATGACAT TATAGGATAG CAAATTGCAA AGGTAATGAA ACCTGCCAGG	629
TAGGTACTAT GTGTCTGGAG TGCTTCCAAA GGACCATTGC TCAGAGGAAT ACTTTGCCAC	689
TACAGGGCAA TTTAATGACA AATCTCAAAT GCAGCAAATT ATTCTCTCAT GAGATGCATG	749
ATGGTTTTTT TTTTTTTTTT TAAAGAAACA AACTCAAGTT GCACTATTGA TAGTTGATCT	809
ATACCTCTAT ATTTCACTTC AGCATGGACA CCTTCAAAC TGCAGCACTTT TTGACAAACA	869
TCAGAAATGT TAATTTATAC CAAGAGAGTA ATTATGCTCA TATTAATGAG ACTCTGGAGT	929
GCTAACAATA AGCAGTTATA ATTAATTATG TAAAAAATGA GAATGGTGAG GGGAAATTGCA	989

TTTCATTATT AAAACAAGG CTAGTTCTTC CTTTAGCATG GGAGCTGAGT GTTTGGGAGG 1049
GTAAGGACTA TAGCAGAATC TCTTCAATGA GCTTATTCTT TATCTTAGAC AAAACAGATT 1109
GTCAAGCCAA GAGCAAGCAC TTGCCTATAA ACCAAGTGCT TTCTCTTTTG CATTTTGAAC 1169
AGCATTGGTC AGGGCTCATG TGTATTGAAT CTTTAAACC AGTAACCCAC GTTTTTTTTC 1229
TGCCACATTT GCGAAGCTTC AGTGCAGCCT ATAACTTTTC ATAGCTTGAG AAAATTAAGA 1289
GTATCCACTT ACTTAGATGG AAGAAGTAAT CAGTATAGAT TCTGATGACT CAGTTTGAAG 1349
CAGTGTTTCT CAACTGAAGC CCTGCTGATA TTTAAGAAA TATCTGGATT CCTAGGCTGG 1409
ACTCCTTTTT GTGGGCAGCT GTCCTGCGCA TTGTAGAATT TTGGCAGCAC CCCTGGACTC 1469
TAGCCACTAG ATACCAATAG CAGTCCTTCC CCCATGTGAC AGCCAAAAAT GTCTTCAGAC 1529
ACTGTCAAAT GTCGCCAGGT GGCAAAATCA CTCCTGGTTG AGAACAGGGT CATCAATGCT 1589
AAGTATCTGT AACTATTTTA ACTCTCAAAA CTGTGATAT ACAAAGTCTA AATTATTAGA 1649
CGACCAATAC TTTAGGTTTA AAGGCATACA AATGAAACAT TCAAAAATCA AAATCTATTC 1709
TGTTTCTCAA ATAGTGAATC TTATAAAATT AATCACAGAA GATGCAAATT GCATCAGAGT 1769
CCCTTAAAT TCCTCTTCGT ATGAGTATTT GAGGGAGGAA TTGGTGATAG TTCCTACTTT 1829
CTATTGGATG GTACTTTGAG ACTCAAAAGC TAAGCTAAGT TGTGTGTGTG TCAGGGTGCG 1889
GGGTGTGGAA TCCCATCAGA TAAAAGCAAA TCCATGTAAT TCATTCAGTA AGTTGTATAT 1949
GTAGAAAAAT GAAAAGTGGG CTATGCAGCT TGGAACTAG AGAATTTTGA AAAATAATGG 2009
AAATCACAAG GATCTTTCTT AAATAAGTAA GAAAATCTGT TTGTAGAATG AAGCAAGCAG 2069
GCAGCCAGAA GACTCAGAAC AAAAGTACAC ATTTTACTCT GTGTACACTG GCAGCACAGT 2129
GGGATTTATT TACCTCTCCC TCCCTAAAAA CCCACACAGC GGTTCTCTT GGGAAATAAG 2189
AGGTTTCCAG CCCAAAGAGA AGGAAAGACT ATGTGGTGTT ACTCTAAAAA GTATTTAATA 2249
ACCGTTTTGT TGTGCTGTT GCTGTTTTGA AATCAGATTG TCTCCTCTCC ATATTTTATT 2309
TACTTCATTC TGTTAATTCC TGTGGAATTA CTTAGAGCAA GCATGGTGAA TTCTCAACTG 2369
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115

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180

185

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190

195

200

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205

210

215

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Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu

220

225

230

235

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240

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Asp Ile Asp Leu Cys

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Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala	
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305 310 315 320	
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Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe	
325 330 335	
CCC AAA ACT GTC ACT CAG AGT CTA AAG AAG ACC ATC AGG TTC CTT CAC	9262
Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His	
340 345 350	
AGC TTC ACA ATG TAC AAA TTG TAT CAG AAG TTA TTT TTA GAA ATG ATA	9310
Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile	
355 360 365	

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請 求 の 範 囲

1. 次の物理化学的性質をもち、破骨細胞の分化及び／又は成熟抑制活性のある蛋白質。
 - (a) 分子量 (SDS-PAGEによる) ; 約60kD (還元条件下)、約60kD及び約120 kD (非還元条件下)
 - (b) 親和性 ; 陽イオン交換体及びヘパリンに親和性を有する。
 - (c) 熱安定性 ; 70℃、10分間又は56℃、30分間の加熱処理により破骨細胞の分化・成熟抑制活性が低下し、90℃、10分間の加熱処理により破骨細胞の分化・成熟抑制活性が失われる。
 - (d) アミノ酸配列 ; 内部アミノ酸配列として配列表 配列番号 1～3 のアミノ酸配列をもつ。
2. N末端配列が配列表 配列番号 7 のアミノ酸配列で示される、請求項 1 記載の蛋白質。
3. ヒト線維芽細胞が産生する、請求項 1 記載の蛋白質。
4. ヒト線維芽細胞を細胞培養し、培養液をイオン交換カラム、アフィニティークラム及び逆相カラムへの吸着及び溶出を行なって精製することを特徴とする請求項 1～3 のいずれかに記載の蛋白質の製造方法。
5. アルミナセラミック片を担体として使用して細胞培養を行なう請求項 4 記載の蛋白質の製造方法。
6. 配列表 配列番号 4 のアミノ酸配列で示される蛋白質。
7. 配列表 配列番号 4 で示されるアミノ酸配列をコードする cDNA。
8. 配列表 配列番号 6 の塩基配列で示される cDNA。
9. 配列表 配列番号 6 の塩基配列で示される cDNA と比較的温和な条件下でハイブリダイズする DNA。
10. 配列表 配列番号 4 で示されるアミノ酸配列をコードする cDNA が発現された蛋白質。
11. 配列表 配列番号 4 で示されるアミノ酸配列と80%以上の相同性を有するア

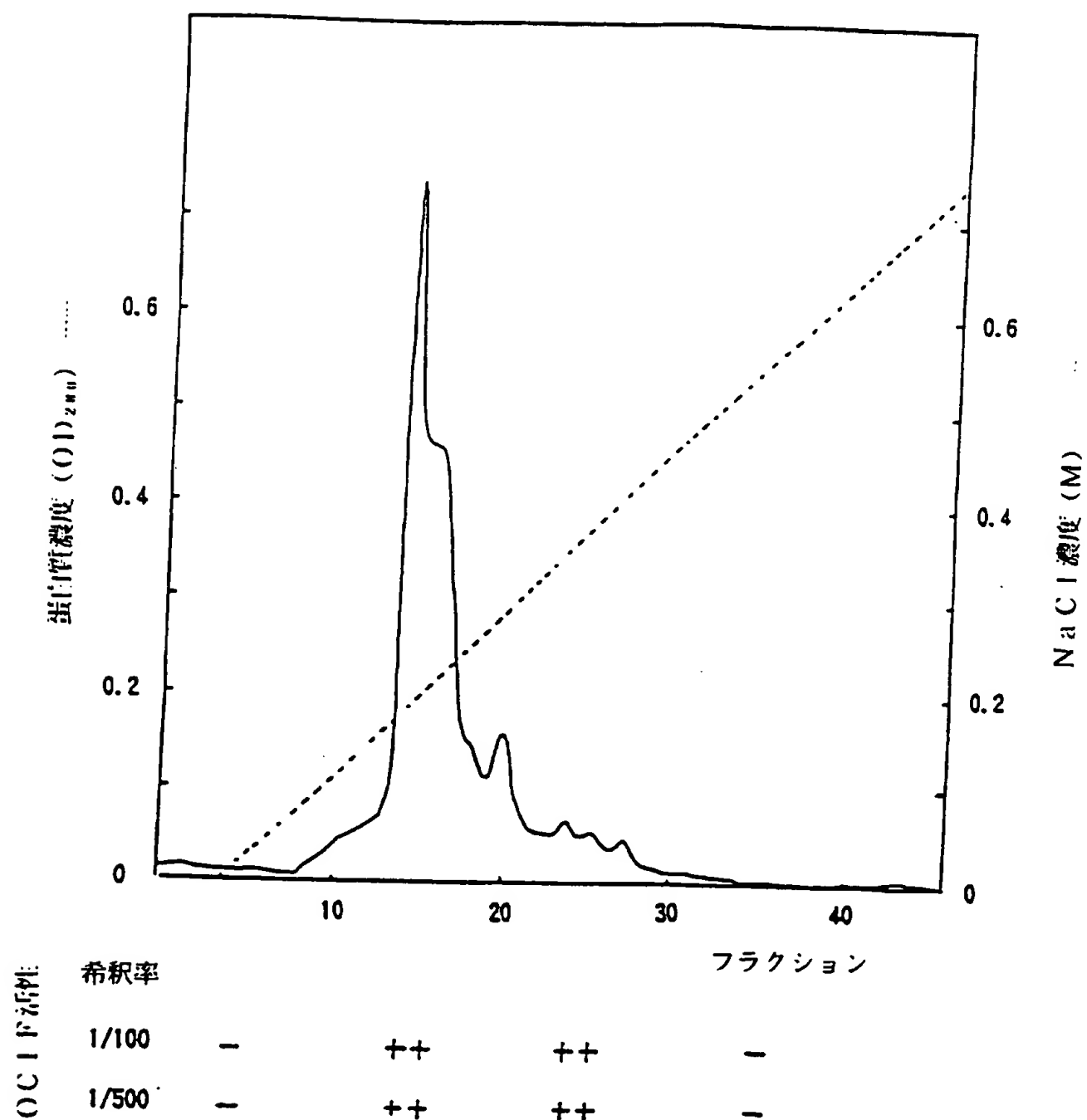
23. 配列表 配列番号 13 で示されるアミノ酸配列をコードする cDNA。
24. 配列表 配列番号 14 の塩基配列で示される cDNA。
25. 配列表 配列番号 14 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
26. 配列表 配列番号 15 で示されるアミノ酸配列をコードする cDNA。
27. 配列表 配列番号 83 の塩基配列で示される cDNA。
28. 配列表 配列番号 83 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
29. 配列表 配列番号 62 で示されるアミノ酸配列をコードする cDNA。
30. 配列表 配列番号 84 の塩基配列で示される cDNA。
31. 配列表 配列番号 84 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
32. 配列表 配列番号 63 で示されるアミノ酸配列をコードする cDNA。
33. 配列表 配列番号 85 の塩基配列で示される cDNA。
34. 配列表 配列番号 85 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
35. 配列表 配列番号 64 で示されるアミノ酸配列をコードする cDNA。
36. 配列表 配列番号 86 の塩基配列で示される cDNA。
37. 配列表 配列番号 86 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
38. 配列表 配列番号 65 で示されるアミノ酸配列をコードする cDNA。
39. 配列表 配列番号 87 の塩基配列で示される cDNA。
40. 配列表 配列番号 87 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
41. 配列表 配列番号 66 で示されるアミノ酸配列をコードする cDNA。
42. 配列表 配列番号 88 の塩基配列で示される cDNA。
43. 配列表 配列番号 88 の塩基配列で示される cDNA を発現することにより得られる蛋白質。

44. 配列表 配列番号 67 で示されるアミノ酸配列をコードする cDNA。
45. 配列表 配列番号 89 の塩基配列で示される cDNA。
46. 配列表 配列番号 89 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
47. 配列表 配列番号 68 で示されるアミノ酸配列をコードする cDNA。
48. 配列表 配列番号 90 の塩基配列で示される cDNA。
49. 配列表 配列番号 90 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
50. 配列表 配列番号 69 で示されるアミノ酸配列をコードする cDNA。
51. 配列表 配列番号 91 の塩基配列で示される cDNA。
52. 配列表 配列番号 91 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
53. 配列表 配列番号 70 で示されるアミノ酸配列をコードする cDNA。
54. 配列表 配列番号 92 の塩基配列で示される cDNA。
55. 配列表 配列番号 92 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
56. 配列表 配列番号 71 で示されるアミノ酸配列をコードする cDNA。
57. 配列表 配列番号 93 の塩基配列で示される cDNA。
58. 配列表 配列番号 93 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
59. 配列表 配列番号 72 で示されるアミノ酸配列をコードする cDNA。
60. 配列表 配列番号 94 の塩基配列で示される cDNA。
61. 配列表 配列番号 94 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
62. 配列表 配列番号 73 で示されるアミノ酸配列をコードする cDNA。
63. 配列表 配列番号 95 の塩基配列で示される cDNA。
64. 配列表 配列番号 95 の塩基配列で示される cDNA を発現することにより得られる蛋白質。

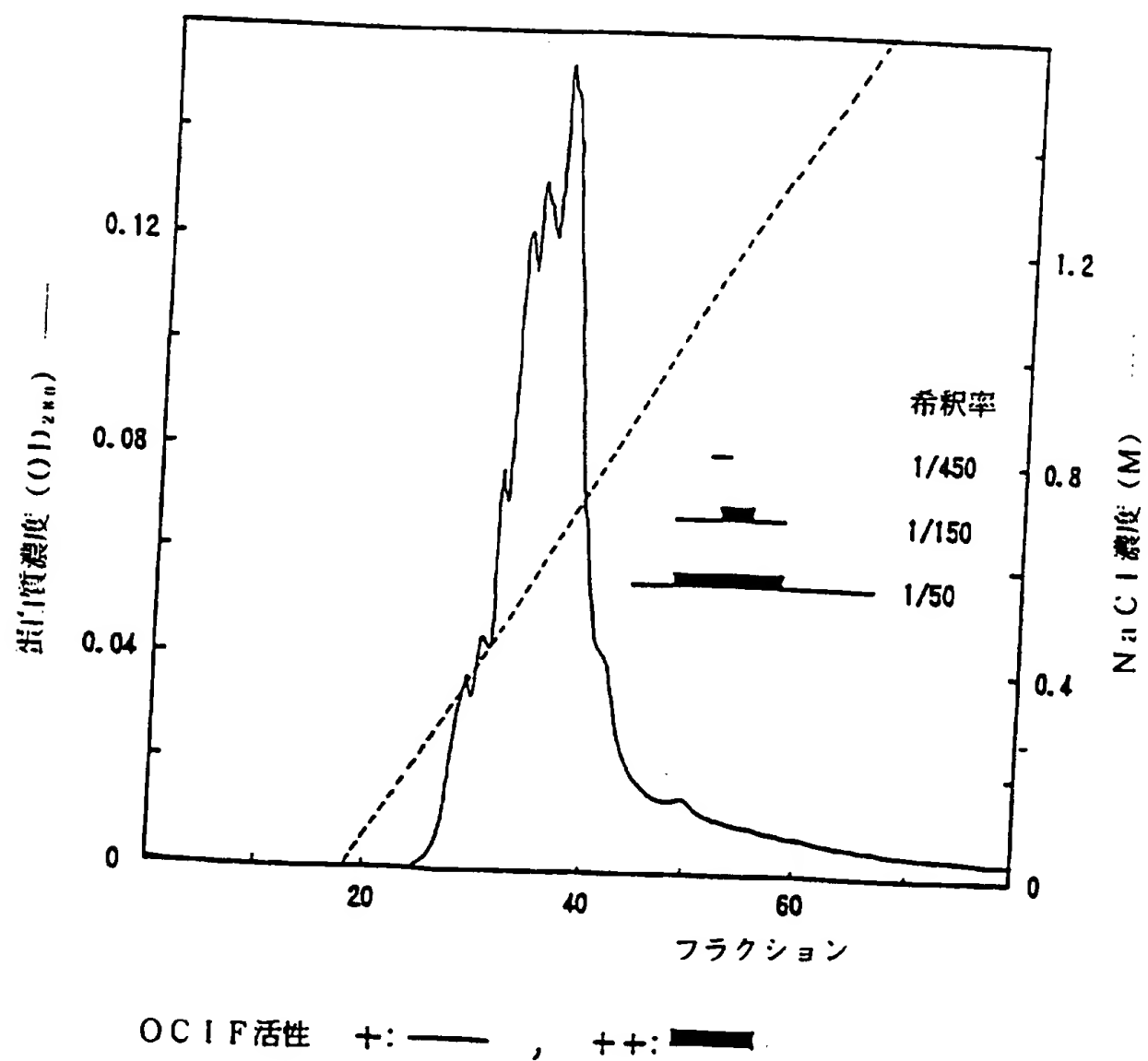
65. 配列表 配列番号 74 で示されるアミノ酸配列をコードする cDNA。
66. 配列表 配列番号 96 の塩基配列で示される cDNA。
67. 配列表 配列番号 96 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
68. 配列表 配列番号 75 で示されるアミノ酸配列をコードする cDNA。
69. 配列表 配列番号 97 の塩基配列で示される cDNA。
70. 配列表 配列番号 97 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
71. 配列表 配列番号 76 で示されるアミノ酸配列をコードする cDNA。
72. 配列表 配列番号 98 の塩基配列で示される cDNA。
73. 配列表 配列番号 98 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
74. 配列表 配列番号 77 で示されるアミノ酸配列をコードする cDNA。
75. 配列表 配列番号 99 の塩基配列で示される cDNA。
76. 配列表 配列番号 99 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
77. 配列表 配列番号 78 で示されるアミノ酸配列をコードする cDNA。
78. 配列表 配列番号 100 の塩基配列で示される cDNA。
79. 配列表 配列番号 100 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
80. 配列表 配列番号 79 で示されるアミノ酸配列をコードする cDNA。
81. 配列表 配列番号 101 の塩基配列で示される cDNA。
82. 配列表 配列番号 101 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
83. 配列表 配列番号 80 で示されるアミノ酸配列をコードする cDNA。
84. 配列表 配列番号 102 の塩基配列で示される cDNA。
85. 配列表 配列番号 102 の塩基配列で示される cDNA を発現することにより得られる蛋白質。

86. 配列表 配列番号 81 で示されるアミノ酸配列をコードする cDNA。
87. 配列表 配列番号 103 の塩基配列で示される cDNA。
88. 配列表 配列番号 103 の塩基配列で示される cDNA を発現することにより得られる蛋白質。
89. 配列表 配列番号 82 で示されるアミノ酸配列をコードする cDNA。
90. 配列表 配列番号 4 のアミノ酸配列をコードするゲノム DNA。
91. 配列表 配列番号 104 及び 105 の塩基配列で示される、請求項 90 記載のゲノム DNA。
92. ヒト破骨細胞形成抑制因子に対し、特異的親和性を示す抗体。
93. 抗体がポリクローナル抗体である、請求項 92 記載の抗体。
94. 抗体がモノクローナル抗体である、請求項 92 記載の抗体。
95. 分子量約 150,000、サブクラス IgG₁、IgG_{2a} 或いは IgG_{2b} である、請求項 95 記載のモノクローナル抗体。
96. 請求項 92～95 記載の抗体を用いることを特徴とする、ヒト破骨細胞形成抑制因子の測定方法。

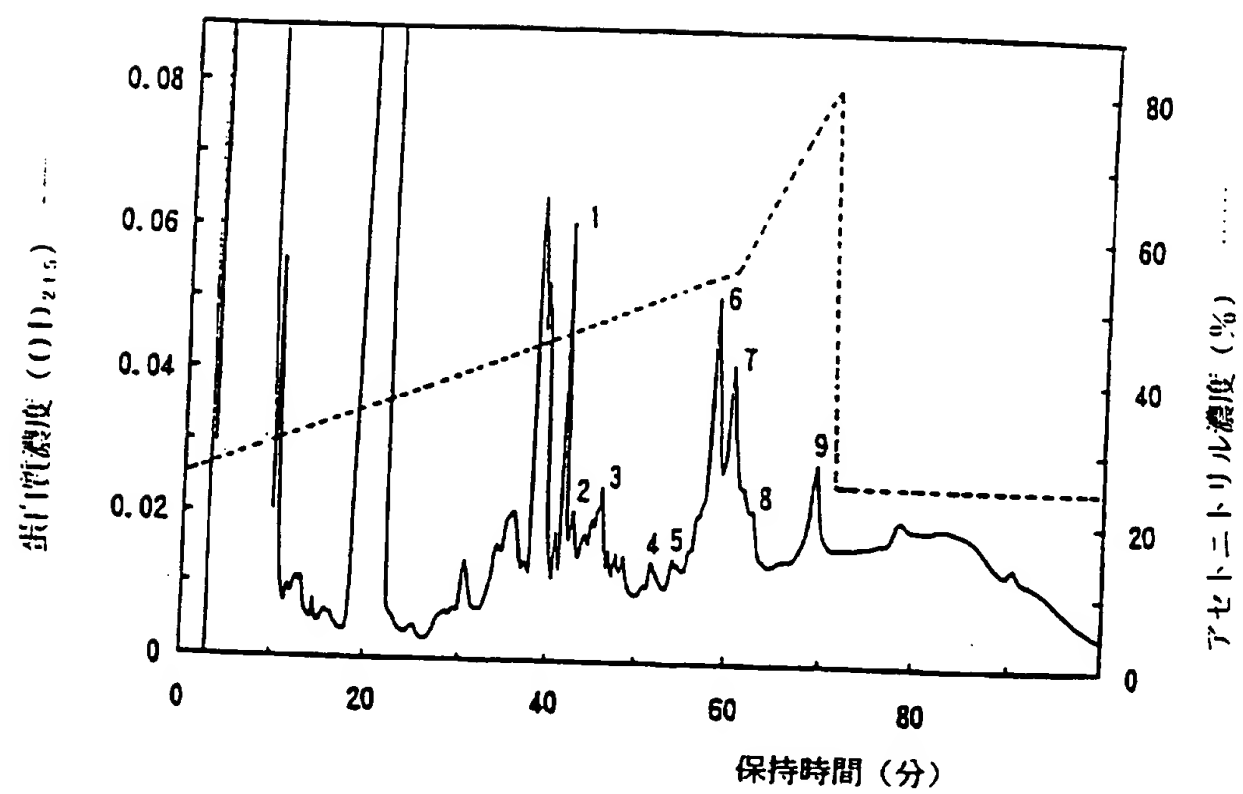
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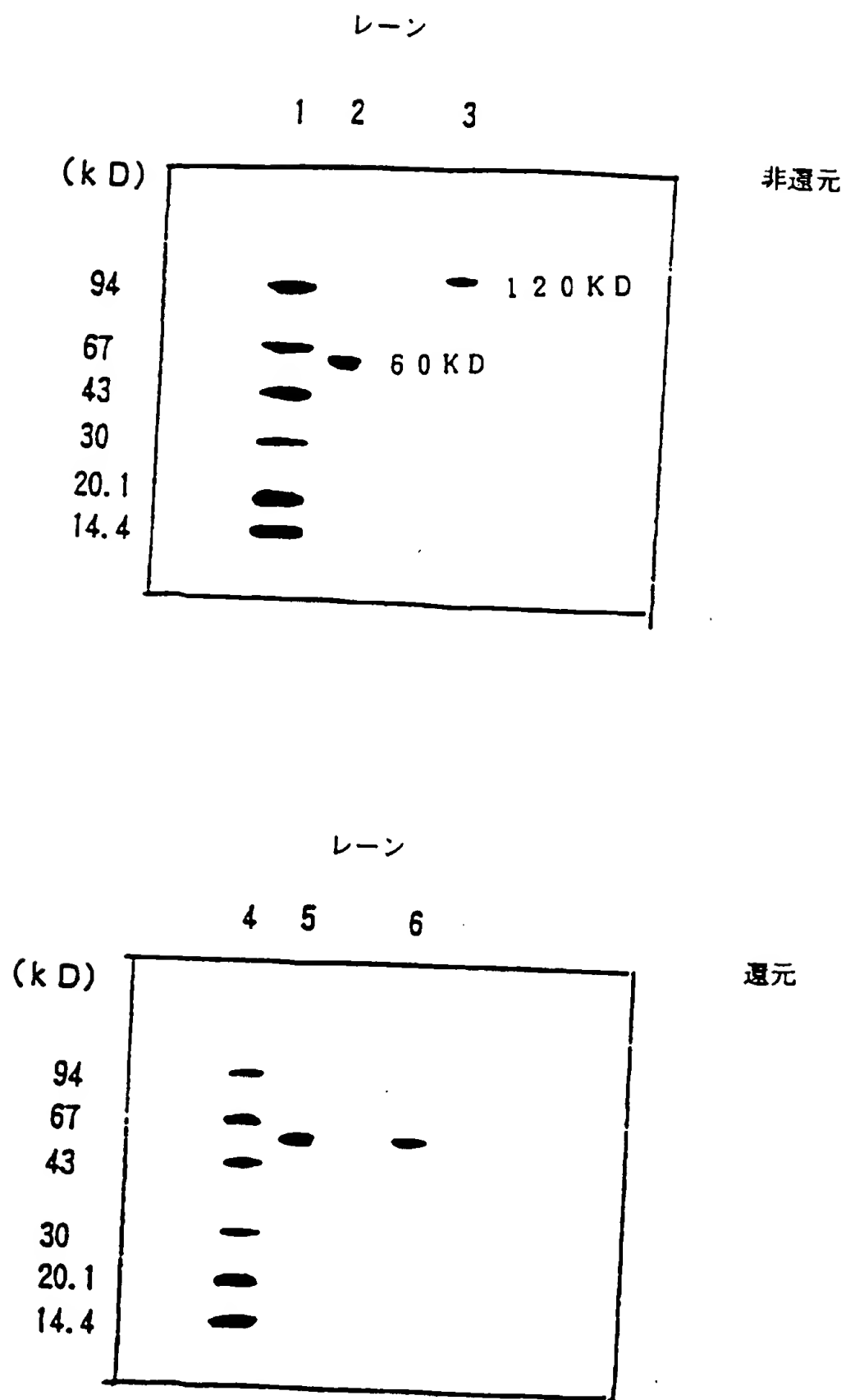
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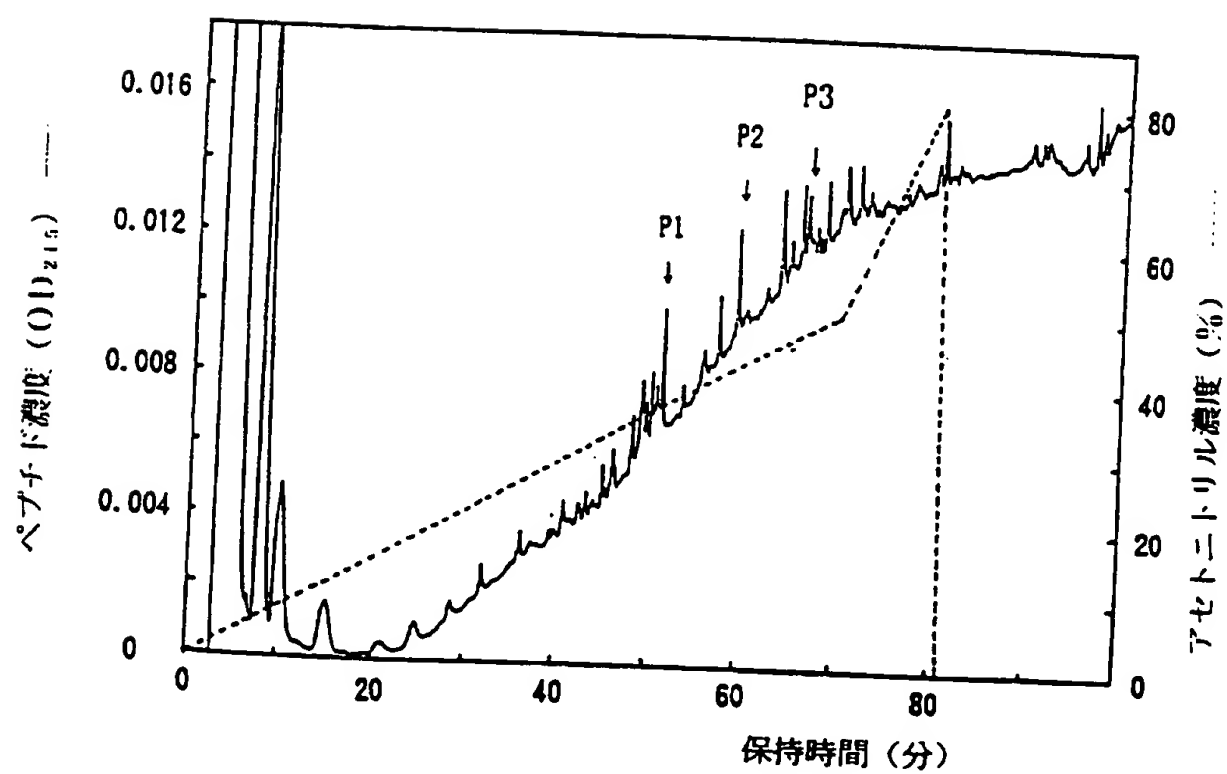
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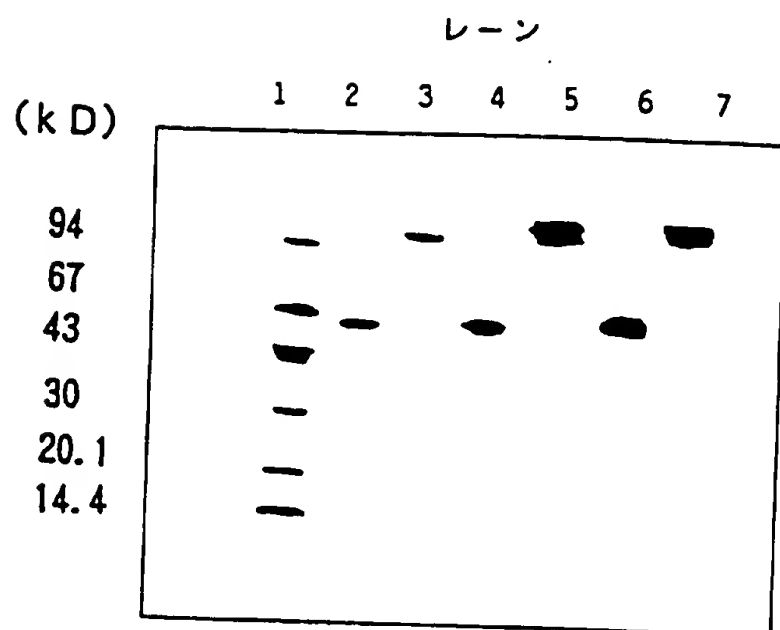
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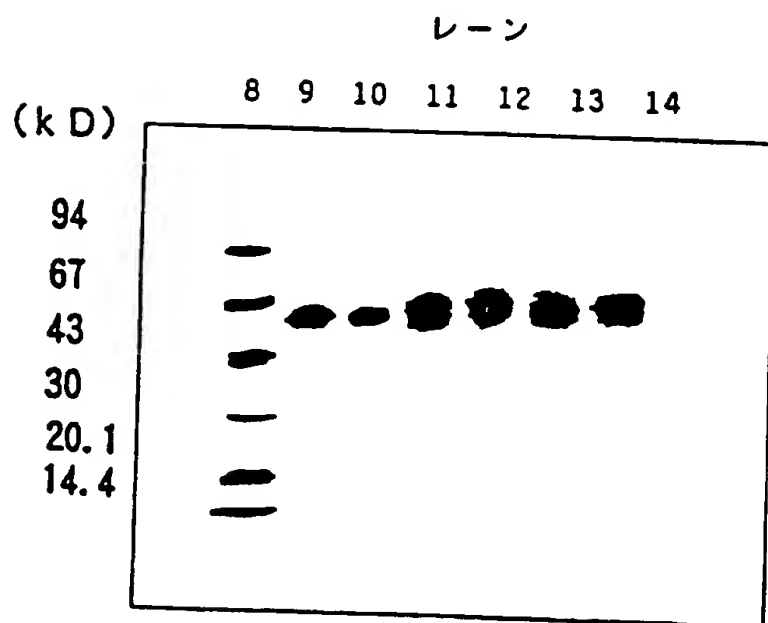
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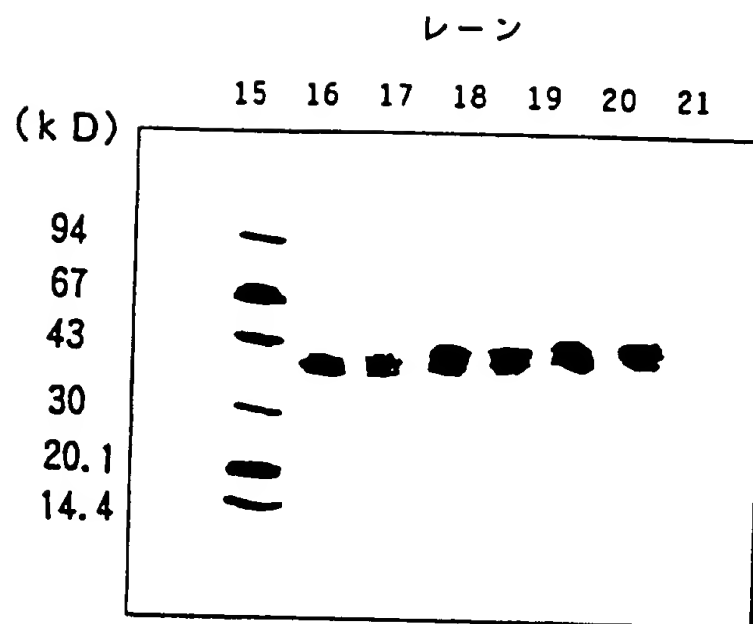
第 6 図



第 7 図



第 8 図



第 9 図

1
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 MNLLCCALVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF2)
 1

61
 VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)

 VCAPCPDHYYTDSWHTSDECLYCSPVCKE-----CNRTHNRVCECKEGRYLEIEFCLK (OCIF2)
 61

121
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 HRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTQKGNAT (OCIF2)
 114

181
 HDNICSGNSESTQKCGIDVTLCEEAFRFAVPTKFTPNWLSVLVDNLPGTKVNAESVERI (OCIF1)

 HDNICSGNSESTQKCGIDVTLCEEAFRFAVPTKFTPNWLSVLVDNLPGTKVNAESVERI (OCIF2)
 174

241
 KRQHSSQEQTQLLKLWKHQNKDQDIVKKIIQDIDLCENSVQRHIGHANLTFEQLRSLME (OCIF1)

 KRQHSSQEQTQLLKLWKHQNKDQDIVKKIIQDIDLCENSVQRHIGHANLTFEQLRSLME (OCIF2)
 234

301
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 SLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALKHISKTYHFPKT (OCIF2)
 294

361
 VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF1)

 VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF2)
 354

第 1 〇 図

1
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 1

61
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121
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 121

181
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 HDNICSGNSESTQKCGIDVTLCEEAFRFAVPTKFTPNWLSVLVDNLPGTKVNAESVERI (OCIF3)
 181

241
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 KRQHSSQEQT FQLKLWKHQNKDQDIVKKIIQDIDLCENSVQRHIGHANLS----- (OCIF3)
 241

301
 SLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALKH SKTYHFPKT (OCIF1)

 -----LWRIKNGDQDTLKGLMHALKH SKTYHFPKT (OCIF3)
 292

361
 VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF1)

 VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF3)
 322

第 1 1 図

1
 MNLLCCALVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF1)
 ** *****
 MNLLCCSLVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF4)
 1

61
 VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)

 VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF4)
 61

121
 HRSCPPGFGVVQAGTPERN TVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTQKGNAT (OCIF1)

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 121

第 1 2 図

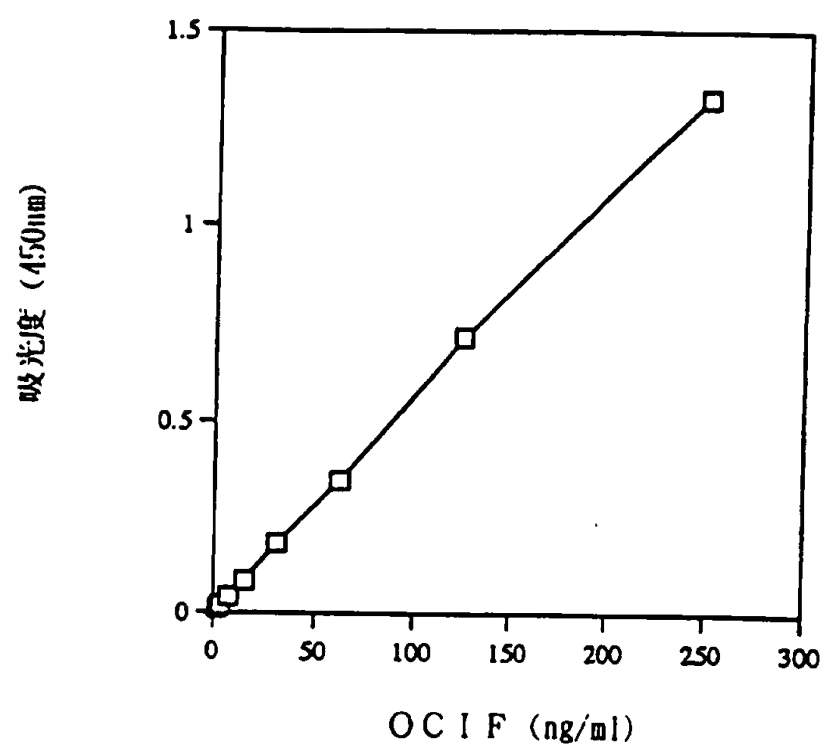
1
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 ** *****
 MNLLCCALVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF5)
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61
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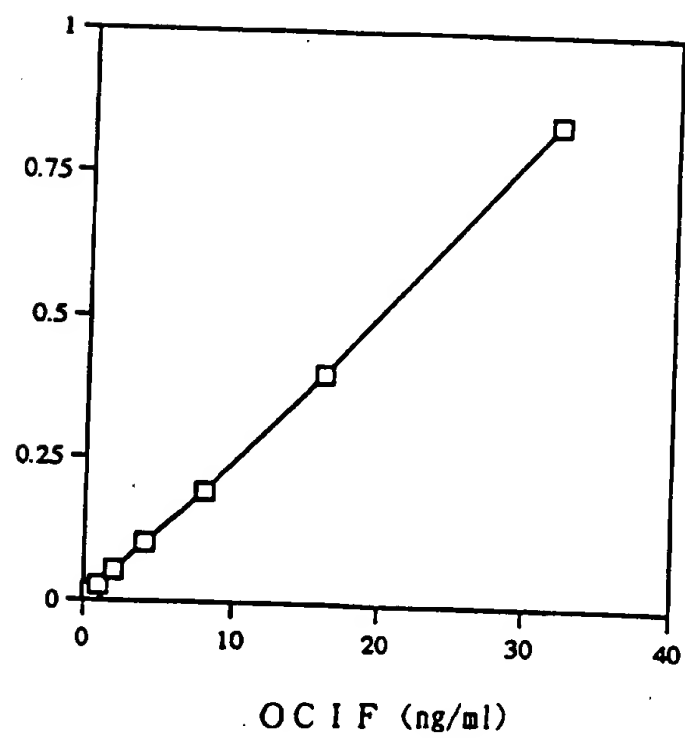
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 61

121
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 ***** *
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 121

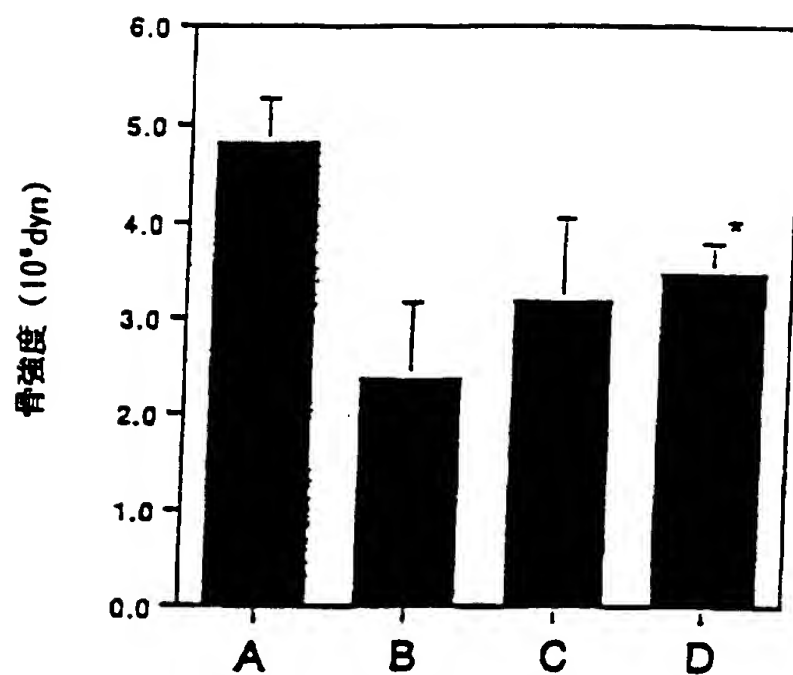
第 1 3 図



第 1 4 図



第 1 5 図



- A : 正常
B : 神経切除+溶媒
C : 神経切除+OCIF 10 μ g/kg/day
D : 神経切除+OCIF 100 μ g/kg/day

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP96/00374

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl⁶ C07K14/52, C07K16/24, C12N15/19, C12N15/06, C12N5/08,
C12N5/10, C12N5/20, C12P21/02, C12P21/08, G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl⁶ C07K14/52, C07K16/24, C12N15/19, C12N15/06, C12N5/08,
C12N5/10, C12N5/20, C12P21/02, C12P21/08, G01N33/577

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS PREVIEWS, CAS ONLINE, WPI, WPI/L

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Fawthrop, F.W. et al. "The effect of transforming growth factor beta on the plasminogen activator activity of normal human osteoblast-like cells and a human osteosarcoma cell line MG-63", J. Bone. Miner. Res. (1992) Vol. 7, No. 12, p. 1363-1371	1 - 96
A	Fenton, A.J. et al. "Long-term culture of disaggregated rat osteoclasts inhibition of bone resorption and reduction of osteoclast-like cell number by calcitonin and PTHrP107-139", J. Cell Physiol. (1993) Vol. 155, No. 1, p. 1-7	1 - 96

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

May 14, 1996 (14. 05. 96)

Date of mailing of the international search report

May 28, 1996 (28. 05. 96)

Name and mailing address of the ISA/

Japanese Patent Office

Facsimile No.

Authorized officer

Telephone No.

A. 発明の属する分野の分類 (国際特許分類 (IPC))

Int. Cl⁸ C07K 14/52, C07K 16/24, C12N 15/19, C12N 15/06, C12N 5/08, C12N 5/10, C12N 5/20, C12P 21/02, C12P 21/08, G01N 33/577

B. 調査を行った分野

調査を行った最小限資料 (国際特許分類 (IPC))

Int. Cl⁸ C07K 14/52, C07K 16/24, C12N 15/19, C12N 15/06, C12N 5/08, C12N 5/10, C12N 5/20, C12P 21/02, C12P 21/08, G01N 33/577

最小限資料以外の資料で調査を行った分野に含まれるもの

国際調査で使用した電子データベース (データベースの名称、調査に使用した用語)

BIOSIS PREVIEWS, CAS ONLINE, WPI, WPI/L

C. 関連すると認められる文献

引用文献の カテゴリー*	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する 請求の範囲の番号
A	Fawthrop, F. W. et al. "The effect of transforming growth factor beta on the plasminogen activator activity of normal human osteoblast-like cells and a human osteosarcoma cell line MG-63", J. Bone Miner. Res. [1992] 第7巻, 第12号, p. 1363-1371	1 - 96 1 - 96
A	Fenton, A. J. et al. "Long-term culture of disaggregated rat osteoclasts inhibition of bone resorption and reduction of osteoclast-like cell number by calcitonin and PTHrP107-139", J. Cell Physiol. [1993] 第155巻, 第1号, p. 1-7	

☐ C欄の続きにも文献が列挙されている。

☐ パテントファミリーに関する別紙を参照。

* 引用文献のカテゴリー

「A」 特に関連のある文献ではなく、一般的技術水準を示すもの

「E」 先行文献ではあるが、国際出願日以後に公表されたもの

「L」 優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献 (理由を付す)

「O」 口頭による開示、使用、展示等に言及する文献

「P」 国際出願日前で、かつ優先権の主張の基礎となる出願

の日の後に公表された文献

「T」 国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの

「X」 特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの

「Y」 特に関連のある文献であって、当該文献と他の1以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの

「&」 同一パテントファミリー文献

国際調査を完了した日

14. 05. 96

国際調査報告の発送日 28.05.96

国際調査機関の名称及びあて先

日本国特許庁 (ISA/JP)

郵便番号 100

東京都千代田区霞が関三丁目4番3号

特許庁審査官 (権限のある職員)

齊藤 真由美

印

4B

9452

電話番号 03-3581-1101 内線 3449

ENGLISH-LANGUAGE TRANSLATION OF

WO 96/26217

SPECIFICATION

NOVEL PROTEINS AND METHODS FOR PRODUCING THE PROTEINS

Field of the invention

This invention relates to a novel protein, osteoclastogenesis inhibitory factor (OCIF), and methods for producing the protein.

Background of the invention

Human bones are always remodelling by the repeated process of resorption and reconstitution. In the process, osteoblasts and osteoclasts are considered to be the cells mainly responsible for bone formation and bone resorption, respectively. A typical example of disease caused by the progression of abnormal bone metabolism is osteoporosis. The disease is known to be provoked by the condition in which bone resorption by osteoclasts exceeds bone formation by osteoblasts, but the mechanism of osteoporosis has not yet been completely elucidated. Osteoporosis causes pain in the bone and makes the bone fragile, leading to fracture. Since osteoporosis increases the number of bedridden old people, it has become a social issue with the increasing number of old people. Therefore, efficacious drugs for the treatment of the disease are expected to be developed. Bone mass reduction caused by the abnormal bone metabolism is thought to be prevented by inhibiting bone resorption, improving bone formation, or improving the balanced metabolism.

Bone formation is expected to be promoted by stimulating growth, differentiation, or activation of osteoblasts. Many cytokines are reported to stimulate growth or differentiation of osteoblasts, i.e. fibroblast growth

factor (FGF) (Rodan S.B. et al., Endocrinology vol.121, p1917, 1987), insulin-like growth factor-I (IGF-I) (Hock J.M. et al., Endocrinology vol. 122, p254, 1988), insulin-like growth factor-II (IGF-II) (McCarthy T. et al., Endocrinology vol.124, p301, 1989), Activin A (Centrella M. et al., Mol, Cell, Biol. vol. 11, p250, 1991), Vasculotropin (Varonique M et al., Biochem. Biophys. Res. Commun. vol. 199, p380, 1994), and bone morphogenetic protein (BMP) (Yamaguchi, A et al., J. Cell Biol. vol. 113, p682, 1991, Sampath T.K. et al., J. Biol Chem. vol.267, p20532, 1992, and Knutsen R. et al., Biochem. Biophys. Res. Commun. vol.194, p1352, 1993.

On the other hand, cytokines which inhibits differentiation and/or maturation of osteoclasts have been paid attention and have been intensively studied. Transforming growth factor- β (Chenu C. et al., Proc. Natl. Acad. Sci. USA, vol.85, p5683, 1988) and interleukin-4 (Kasano K. et al., Bone-Miner., vol. 21, p179, 1993) are found to inhibit the differentiation of osteoclasts. Calcitonin (Bone-Miner., vol.17, p347, 1992), Macrophage colony-stimulating factor (Hattersley G. et al. J. Cell. Physiol. vol.137, p199, 1988), interleukin-4 (Watanabe, K. et al., Biochem. Biophys. Res. Commun. vol. 172, p1035, 1990), and interferon- γ (Gowen M. et al., J. Bone Miner. Res., vol.1, p469, 1986) are found to inhibit bone resorption by osteoclasts.

These cytokines are expected to be efficacious drugs for improving bone mass reduction by stimulating bone formation and/or by inhibiting bone resorption. The cytokines such as insulin like growth factor-I and bone morphogenetic proteins are now investigated in clinical trials for their

effects in treatment of patients with bone diseases. Calcitonin is already used as a drug to care osteoporosis and to diminish pain in osteoporosis.

Examples of drugs now clinically utilized for the treatment of bone diseases and for shortening the treatment period are dihydroxyvitamine D₃, vitamin K₂, calcitonin and its derivatives, hormones such as estradiol, ipriflavon, and calcium preparations. However, these drugs do not provide satisfactory therapeutic effects, and novel drug substances have been expected to be developed. As mentioned, bone metabolism is controlled in the balance between bone resorption and bone formation. Therefore, cytokines which inhibit osteoclast differentiation and/or maturation are expected to be developed as drugs for the treatment of bone diseases such as osteoporosis.

Disclosure of Invention

This invention was initiated from the view point described above. The purpose of this invention is to offer both a novel factor termed osteoclastogenesis inhibitory factor (OCIF) and a procedure to produce the factor efficiently.

The inventors have intensively searched for osteoclastogenesis inhibitory factors in human embryonic fibroblast IMR-90 (ATCC CCL186) conditioned medium and have found a novel osteoclastogenesis inhibitory factor (OCIF) which inhibits differentiation and/or maturation of osteoclasts.

The inventors have established a method for accumulating the protein to a high concentration by culturing IMR-90 cells using alumina ceramic pieces as the cell adherence matrices.

The inventors have also established an efficient method for isolating the protein, OCIF, from the IMR-90 conditioned medium using the following sequential column chromatography, ion-exchange, heparin affinity, cibacron-blue affinity, and reverse phase.

The inventors, based on the amino acid sequence of the purified natural OCIF, successfully cloned a cDNA encoding this protein. The inventors established also a procedure to produce this protein which inhibits differentiation of osteoclasts. This invention concerns a protein which is produced by human lung fibroblast cells, has molecular weights in SDS-PAGE of 60 KD in the reducing conditions and 120 KD under the non-reducing conditions, has affinity for both cation-exchange resins and heparin, reduces its activity to inhibit differentiation and maturation of osteoclasts if treated for 10 minutes at 70 °C or for 30 minutes at 56 °C, and lose its activity to inhibit differentiation and maturation of osteoclasts by the treatment for 10 minutes at 90 °C. The amino acid sequence of the protein OCIF which is described in the present invention is clearly different from any of know factors inhibiting formation of osteoclasts.

The invention includes a method to purify OCIF protein, comprising ; (1) culturing human fibroblasts, (2) applying the conditioned medium to a heparin column to obtain the adsorbed fraction, (3) purifying the OCIF protein using a cation-exchange column, (4) purifying the OCIF protein using a heparin affinity column, (5) purifying the OCIF protein using a cibacron blue affinity column, (6) isolating the OCIF protein using reverse-phase column chromatography. Cibacron blue F3GA coupled to a carrier made of synthetic

hydrophilic polymers is an example of materials used to prepare Cibacron blue columns. These columns are conventionally called "blue columns".

The invention includes a method for accumulating the OCIF protein to a high concentration by culturing human fibroblasts using alumina ceramic pieces as the cell-adherence matrices.

Moreover, the inventors determined the amino acid sequences of the peptides derived from OCIF, designed the primers based on these amino acid sequences, and obtained cDNA fragments encoding OCIF from a cDNA library of IMR-90 cells.

Detailed description of the invention

The OCIF protein of the present invention can be isolated from human fibroblast conditioned medium with high yield. The procedure to isolate OCIF is based on ordinary techniques for purifying proteins from biomaterials, in accordance with the physical and chemical properties of OCIF protein. For example, concentrating procedure includes ordinary biochemical techniques such as ultrafiltration, lyophilization, and dialysis. Purifying procedure includes combinations of several chromatographic techniques for purifying proteins such as ion-exchange column chromatography, affinity column chromatography, gel filtration column chromatography, hydrophobic column chromatography, reverse phase column chromatography, and preparative gel electrophoresis. The human fibroblast used for production of the OCIF protein is preferably IMR-90. A method for producing the IMR-90 conditioned medium is preferably a process comprising, adhering human embryonic fibroblast IMR-90 cells to alumina

ceramic pieces in roller-bottles, using DMEM medium supplemented with 5 % new born calf serum for the cell culture, and cultivating the cells in roller-bottles for 7 to 10 days by stand cultivation. CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) is preferably added to the buffer as a detergent in the purification steps of OCIF protein.

OCIF protein of the instant invention can be initially obtained as a heparin binding basic OCIF fraction by applying the culture medium to a heparin column (Heparin-Sepharose CL-6B, Pharmacia), eluting with 10 mM Tris-HCl buffer, pH 7.5, containing 2 M NaCl, and then by applying the OCIF fraction to a Q⁺ anion-exchange column (HiLoad-Q/FF, Pharmacia), and collecting non-adsorbed fraction. OCIF protein can be purified by subjecting the obtained OCIF fraction to purification on a S⁺ cation-exchange column (HiLoad-S/FF, Pharmacia), a heparin column (Heparin-5PW, TOSOH), Cibacrone Blue column (Blue-5PW, TOSOH), and a reverse-phase column (BU-300 C4, Perkin Elmer) and the material is defined by the previously described properties.

The present invention relates to a method of cloning cDNA encoding the OCIF protein based on the amino acid sequence of natural OCIF and a method of obtaining recombinant OCIF protein that inhibits differentiation and/or maturation of osteoclasts. The OCIF protein is purified according to the method described in the present invention and is treated with endopeptidase (for example, lysylendopeptidase). The amino acid sequences of the peptides produced by the digestion are determined and the mixture of oligonucleotides

that can encode each internal amino acid sequence was synthesized. The OCIF cDNA fragment is obtained by PCR (preferably RT-PCR, reverse transcriptase PCR) using the oligonucleotide mixtures described above as primers. The full length OCIF cDNA encoding the OCIF protein is cloned from a cDNA library using the obtained OCIF DNA fragment as a probe. The OCIF cDNA containing the entire coding region is inserted into an expression vector. The recombinant OCIF can be produced by expressing the OCIF cDNA containing the entire coding region in mammalian cells or bacteria.

The present invention relates to the novel proteins OCIF2, OCIF3, OCIF4, and OCIF5 that are variants of OCIF and have the activity described above. These OCIF variants are obtained from the cDNA library constructed with IMR-90 poly(A) + RNA by hybridization using the OCIF cDNA fragment as a probe. Each of the OCIF variant cDNAs containing the entire coding region is inserted into an expression vector. Each recombinant OCIF variant can be produced by expressing each of the OCIF variant cDNAs containing the entire coding region in the conventional hosts. Each recombinant OCIF variant can be purified according to the method described in this invention. Each recombinant OCIF variant has an ability to inhibit osteoclastogenesis.

The present invention further includes OCIF mutants. They are substitution mutants comprising replacement of one cysteine residue possibly involved in dimer formation with serine residue, and various deletion mutants of OCIF. Substitutions or deletions are introduced into the OCIF cDNA using

polymerase chain reaction (PCR) or by restriction enzyme digestion. Each of these mutated OCIF cDNAs is inserted into a vector containing an appropriate promoter for gene expression. The resultant expression vector for each of the OCIF mutants is transfected into eukaryotic cells such as mammalian cells. Each of OCIF mutants can be obtained and purified from the conditioned media of the transfected cells.

The present invention provides polyclonal antibodies and a method to quantitatively determine OCIF concentration using these polyclonal antibodies.

As antigens (immunogens), natural OCIF obtained from IMR-90 conditioned medium, recombinant OCIF produced by such hosts as microorganisms and eukaryotes using OCIF cDNA, synthetic peptides designed based on the amino acid sequence of OCIF, or peptides obtained from OCIF by partial digestion can be used. Anti-OCIF polyclonal antibodies are obtained by immunizing appropriate mammals with the antigens in combination with adjuvants for immunization if necessary, purifying from the serum by the ordinary purification methods. The anti-OCIF polyclonal antibodies which are labelled with radioisotopes or enzymes can be used in radio-immunoassay (RIA) system or immunoassay (EIA) system. By using these assay systems, the concentrations of OCIF in biological materials such as blood and ascites and cells-culture medium can be easily determined.

The antibodies in the present invention can be used in radio immunoassay (RIA) or enzyme immunoassay (EIA). By using these assay systems, the concentration of OCIF in biological materials such as blood and ascites can

be easily determined.

The present invention provides novel monoclonal antibodies and a method to quantitatively determine OCIF concentration using these monoclonal antibodies.

Anti-OCIF monoclonal antibodies can be produced by the conventional method using OCIF as an antigen. Native OCIF obtained from the culture medium of IMR-90 cells and recombinant OCIF produced by such hosts as microorganisms and eukaryotes using OCIF cDNA can be used as antigens. Alternatively, synthesized peptides designed based on the amino acid sequence of OCIF and peptides obtained from OCIF by partial digestion can be also used as antigens. Immunized lymphocytes obtained by immunization of mammals with the antigen or by an in vitro immunization method were fused with myeloma of mammals to obtain hybridoma. The hybridoma clones secreting antibody which recognizes OCIF were selected from the hybridomas obtained by the cell fusion. The desired antibodies can be obtained by cell culture of the selected hybridoma clones. In preparation of hybridoma, small animals such as mice or rats are generally used for immunization. To immunize, OCIF is suitably diluted with a saline solution (0.15 M NaCl), and is intravenously or intraperitoneally administered with an adjuvant to animals for 2 -5 times every 2 -20 days. The immunized animal was killed three days after final immunization, the spleen was taken out and the splenocytes were used as immunized B lymphocytes.

Mouse myeloma cell lines for cell fusion with the immunized B lymphocytes include, for example, p3/x63-Ag8, p3-U1, NS-1, MPC-11, SP-2/0, F0, p3x63

Ag8.653, and S194. Rat R-210 cell line may also be used. Human B lymphocytes are immunized by an in vitro immunization method and are fused with human myeloma cell line or EB virus transformed human B lymphocytes which are used as a parent cell line for cell fusion, to produce human type antibody.

Cell fusion of the immunized B lymphocytes and myeloma cell line is carried out principally by the conventional methods. For example, the method of Koehler G. et al. (Nature 256, 495-497, 1975) is generally used, and also an electric pulse method can be applied to cell fusion. The immunized B lymphocytes and transformed B cells are mixed at conventional ratios and a cell culture medium without FBS containing polyethylene glycol is generally used for cell fusion. The B lymphocytes fused with myeloma cell lines are cultured in HAT selection medium containing FBS to select hybridoma.

For screening of hybridoma producing anti-OCIF antibody, EIA, plaque assay, Ouchterlony, or agglutination assay can be principally used. Among them, EIA is simple and easy to operate with sufficient accuracy and is generally used. By EIA using purified OCIF, the desired antibody can be selected easily and accurately. Thus obtained hybridoma can be cultured by the conventional method of cell culture and frozen for stock if necessary. The antibody can be produced by culturing hybridoma using the ordinary cell culture method or by transplanting hybridoma intraperitoneally to animals. The antibody can be purified by the ordinary purification methods such as salt precipitation, gel filtration, and affinity chromatography. The obtained antibody specifically reacts with OCIF and can be used for determination of OCIF concentration and for purification of OCIF. The antibodies of the

present invention recognize epitopes of OCIF and have high affinity to OCIF. Therefore, they can be used for the construction of EIA. By (using) this assay system, the concentration of OCIF in biological materials such as blood and ascites can be easily determined.

The agents used for treating bone diseases that contain OCIF as an effective ingredient are provided by the present invention. Rats were subjected to denervation of left forelimb. Test compounds were administered daily after surgery for 14 days. After 2 weeks treatment, the animals were sacrificed and their forelimbs were dissected. Thereafter bones were tested for mechanical strength by three point bending method. OCIF improved mechanical strength of bone in a dose dependent manner.

The OCIF protein of the invention is useful as a pharmaceutical ingredients for treating or improving decreased bone mass in such as osteoporosis, bone diseases such as rheumatism, osteoarthritis, and abnormal bone metabolism in multiple myeloma. The OCIF protein is also useful as an antigen to establish immunological diagnosis of the diseases. Pharmaceutical preparations containing the OCIF protein as an active ingredients are formulated and can be orally or parenterally administered. The preparation contains the OCIF protein of the present invention as an efficacious ingredient and is safely administered to human and animals. Examples of the pharmaceutical preparations include compositions for injection or intravenous drip, suppositories, nasal preparations, sublingual preparations, and tapes for percutaneous absorption. The pharmaceutical preparation for injection can

be prepared by mixing the pharmacologically efficacious amount of OCIF protein and pharmaceutically acceptable carriers. The carriers are vehicles and/or activators, e.g. amino acids, saccharides, cellulose derivatives, and other organic and inorganic compounds which are generally added to active ingredients. When the OCIF protein is mixed with the vehicles and/or activators to prepare injections, pH adjuster, buffer, stabilizer, solubilizing agent, etc. can be added, if necessary.

Brief description of the figures

Figure 1 shows the elution pattern of crude OCIF protein (Hiload-Q/FF pass-through fraction ; sample 3) from a Hiload-S/HP column.

Figure 2 shows the elution pattern of crude OCIF protein (heparin-5PW fraction ; sample 5) from a blue-5PW column.

Figure 3 shows the elution pattern of OCIF protein (blue-5PW fraction 49 to 50) from a reverse-phase column.

Figure 4 shows the SDS-PAGE of isolated OCIF proteins under reducing conditions or non-reducing conditions.

Description of the lanes,

lane 1, 4 ; molecular weight marker proteins

lane 2, 5 ; OCIF protein of peak 6 in figure 3

lane 3, 6 ; OCIF protein of peak 7 in figure 3

Figure 5 shows the elution pattern of peptides obtained by the digestion of pyridyl ethylated OCIF protein digested with lysylendopeptidase, on a reverse-phase column.

Figure 6 shows the SDS-PAGE of isolated natural(n) OCIF protein and recombinant(r) OCIF proteins under non-reducing conditions. rOCIF(E) and rOCIF(C) were produced in 293/EBNA cells and in CHO cells, respectively.

Description of the lanes,

lane 1 ; molecular weight marker proteins

lane 2 ; a monomer type nOCIF protein

lane 3 ; a dimer type nOCIF protein

lane 4 ; a monomer type rOCIF(E) protein

lane 5 ; a dimer type rOCIF(E) protein

lane 6 ; a monomer type rOCIF(C) protein

lane 7 ; a dimer type rOCIF(C) protein

Figure 7 shows the SDS-PAGE of isolated natural(n) OCIF proteins and recombinant (r) OCIF proteins under reducing conditions. rOCIF(E) and rOCIF(C) were produced in 293/EBNA cells and in CHO cells, respectively.

Description of the lanes,

lane 8 ; molecular weight marker proteins

lane 9 ; a monomer type nOCIF protein

lane 10 ; a dimer type nOCIF protein

lane 11 ; a monomer type rOCIF(E) protein

lane 12 ; a dimer type rOCIF(E) protein

lane 13 ; a monomer type rOCIF(C) protein

lane 14 ; a dimer type rOCIF(C) protein

Figure 8 shows the SDS-PAGE of isolated natural(n) OCIF proteins and recombinant(r) OCIF proteins from which N-linked sugar chains were removed

under reducing conditions. rOCIF(E) and rOCIF(C) are rOCIF protein produced in 293/EBNA cells and in CHO cells, respectively.

Description of the lanes,

lane 15 ; molecular weight marker proteins

lane 16 ; a monomer type nOCIF protein

lane 17 ; a dimer type nOCIF protein

lane 18 ; a monomer type rOCIF(E) protein

lane 19 ; a dimer type rOCIF(E) protein

lane 20 ; a monomer type rOCIF(C) protein

lane 21 ; a dimer type rOCIF(C) protein

Figure 9 shows comparison of amino acid sequences between OCIF and OCIF2.

Figure 10 shows comparison of amino acid sequences between OCIF and OCIF3.

Figure 11 shows comparison of amino acid sequences between OCIF and OCIF4.

Figure 12 shows comparison of amino acid sequences between OCIF and OCIF5.

Figure 13 shows standard curve for determination of OCIF protein concentration by an EIA employing anti-OCIF polyclonal antibodies.

Figure 14 shows standard curve for determination of OCIF protein concentration by an EIA employing anti-OCIF monoclonal antibodies.

Figure 15 shows the effect of rOCIF protein on osteoporosis.

Best Mode for Carrying Out the Invention

The present invention will be further explained by the following examples, however, the scope of the invention is not restricted to the examples.

EXAMPLE 1

Preparation of a conditioned medium of human fibroblast IMR-90

Human fetal lung fibroblast IMR-90 (ATCC-CCL186) cells were cultured on alumina ceramic pieces (80 g) (alumina: 99.5%, manufactured by Toshiba Ceramic K.K.) in DMEM medium (manufactured by Gibco BRL Co.) supplemented with 5% CS and 10mM HEPES buffer (500 ml/roller bottle) at 37°C under the presence of 5% CO₂ for 7 to 10 days using 60 roller bottles (490 cm², 110 x 171mm, manufactured by Coning Co.) in static culture. The conditioned medium was harvested, and a fresh medium was added to the roller bottles. About 30L of IMR-90 conditioned medium per batch culture was obtained. The conditioned medium was designated as sample 1.

EXAMPLE 2

Assay method for osteoclast development inhibitory activity

Osteoclast development inhibitory activity was assayed by measuring tartrate-resistant acid phosphatase (TRAP) activity according to the methods of M. Kumegawa et.al (Protein • Nucleic Acid • Enzyme, vol.34 p999, 1989) and N. Takahashi et.al (Endocrinology, vol.122, p1373, 1988) with modifications. Briefly, bone marrow cells obtained from 17 day-old mouse were suspended in α -MEM (manufactured by GIBCO BRL Co.) containing 10% FBS, 2×10^{-8} M of activated vitamin D₃, and each test sample, and were inoculated to each well of 96-well plate at a cell density of 3×10^5 cells/0.2 ml/well. The plates were incubated for 7 days at 37°C in humidified 5%CO₂. Cultures were further continued by replacing 0.16 ml of old medium with the same volume of fresh

medium on day 3 and day 5 after starting cultivation. On day 7, after washing the plates with phosphate buffered saline, cells were fixed with ethanol/acetone (1:1) for 1 min. at room temperature, and then osteoclast development was tested by determining for phosphatase activity using a kit (Acid Phosphatase, Leucocyte, Catalog No.387-A, manufactured by Sigma Co.). The decrease of TRAP positive cells was taken as an indication of OCIF activity.

EXAMPLE 3

Purification of OCIF

i) Heparin Sepharose CL-6B column chromatography

The 90L of IMR-90 conditioned medium (sample 1) was filtrated with 0.22 μ membrane filter (hydrophilic Milidisk, 2000 cm^2 , Milipore Co.), and was divided into three portions. Each portion (30 l) was applied to a heparin Sepharose CL-6B column (5 x 4.1 cm, Pharmacia Co.) equilibrated with 10mM Tris-HCl containing 0.3M NaCl, pH 7.5. After washing the column with 10mM Tris-HCl, pH 7.5 at a flow rate of 500 ml/hr., heparin Sepharose CL-6B adsorbent protein fraction was eluted with 10mM Tris-HCl, pH 7.5, containing 2M NaCl. The fraction was designated as sample 2.

ii) HiLoad-Q/FF column chromatography

The heparin Sepharose-adsorbent fraction (sample 2) was dialyzed against 10mM Tris-HCl, pH 7.5, supplemented with CHAPS to a final concentration of 0.1%, incubated at 4 °C overnight, and divided into two portions. Each

portion was then applied to an anion-exchange column (HiLoad-Q/FF, 2.6 x 10 cm, Pharmacia Co.) which was equilibrated with 50mM Tris-HCl, 0.1% CHAPS, pH 7.5 to obtain a non-adsorbent fraction (1000 ml). The fraction was designated as sample 3.

iii) HiLoad-S/HP column chromatography

The HiLoad-Q non-adsorbent fraction (sample 3) was applied to a cation-exchange column (HiLoad-S/HP, 2.6 x 10 cm, Pharmacia Co.) which was equilibrated with 50 mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 50 mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted with linear gradient from 0 to 1 M NaCl at a flow rate of 8 ml/min for 100 min. and fractions (12 ml) were collected. Each ten fractions from number 1 to 40 was pooled to form one portion. Each 100 μ l of the four portions was tested for OCIF activity. OCIF activity was observed in fractions from 11 to 30 (as shown in Figure 1). The fractions from 21 to 30 which had higher specific activity were collected and was designated as sample 4.

iv) Heparin-5PW affinity column chromatography

One hundred and twenty ml of HiLoad-S fraction from 21 to 30 (sample 4) was diluted with 240 ml of 50 mM Tris-HCl, 0.1% CHAPS, pH 7.5, and applied to heparin-5PW affinity column (0.8 x 7.5 cm, Tosoh Co.) which was equilibrated with 50mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 50mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted with linear gradient from 0 to 2M NaCl at a flow rate of 0.5ml/min for 60 min. and fractions

(0.5 ml) were collected. Fifty μ l was removed from each fraction to test for OCIF activity. The active fractions, eluted with 0.7 to 1.3M NaCl was pooled and was designated as sample 5.

v) Blue 5PW affinity column chromatography

Ten ml of sample 5 was diluted with 190 ml of 50mM Tris-HCl, 0.1% CHAPS, pH 7.5 and applied to a blue-5PW affinity column, (0.5x5 cm, Tosoh Co.) which was equilibrated with 50mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 50mM Tris-HCl, 0.1% CHAPS, pH7.5, the adsorbed protein was eluted with a 30 ml linear gradient from 0 to 2M NaCl at a flow rate of 0.5 ml/min., and fractions (0.5 ml) were collected. Using 25 μ l of each fraction, OCIF activity was evaluated. The fractions number 49 to 70, eluted with 1.0-1.6M NaCl had OCIF activity.

vi) Reverse phase column chromatography

The blue 5PW fraction obtained by collecting fractions from 49 to 50 was acidified with 10 μ l of 25% TFA and applied to a reverse phase C4 column (BU-300, 2.1x220mm, manufactured by Perkin-Elmer) which was equilibrated with 0.1% of TFA and 25% of acetonitrile. The adsorbed protein was eluted with linear gradient from 25 to 55% acetonitrile at a flow rate of 0.2 ml/min. for 60 min., and each protein peak was collected (Fig.3). One hundred μ l of each peak fraction was tested for OCIF activity, and peak 6 and the peak 7 had OCIF activity. The result was shown in Table 1.

Table 1

OCIF activity eluted from reverse phase C4 column

Sample	Dilution			
	1/40	1/120	1/360	1/1080
Peak 6	++	++	+	-
Peak 7	++	+	-	-

[++ means OCIF activity inhibiting osteoclast development more than 80%, + means OCIF activity inhibiting osteoclast development between 30% and 80%, and - means no OCIF activity.]

EXAMPLE 4

Molecular weight of OCIF protein

The two protein peaks (6 and 7) with OCIF activity were subjected to SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions. Briefly, 20 μ l of each peak fraction was concentrated under vacuum and dissolved in 1.5 μ l of 10mM Tris-HCl, pH 8, 1mM EDTA, 2.5% SDS, 0.01% bromophenol blue, and incubated at 37°C overnight under non-reducing conditions or under reducing conditions (with 5% of 2-mercaptoethanol). Each 1.0 μ l of sample was then analyzed by SDS-polyacrylamide gel electrophoresis with a gradient gel of 10-15% acrylamide (Pharmacia Co.) and an electrophoresis-device (Fast System, Pharmacia Co.). The following molecular weight marker proteins were used to calculate molecular weight : phosphorylase b (94 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20.0 kD), and lactalbumin (14.4 kD). After

electrophoresis, protein bands were visualized by silver stain using Phast Silver Stain Kit. The results were shown in Fig. 4.

A protein band with an apparent 60 KD was detected in the peak 6 protein under both reducing and non-reducing conditions. A protein band with an apparent 60 KD was detected under reducing conditions and a protein band with an apparent 120 KD was detected under non-reducing conditions in the peak 7 protein. Therefore, the protein of peak 7 was considered to be a homodimer of the protein of peak 6.

EXAMPLE 5

Thermostability of OCIF

Twenty μ l of sample from the blue-5PW fractions 51 and 52 was diluted to 30 μ l with 10 mM phosphate buffered saline, pH 7.2, and incubated for 10 min. at 70°C or 90 °C, or for 30 min. at 56°C. The heat-treated samples were tested for OCIF activity. The results were shown in Table 2.

Table 2

Thermostability of OCIF

Sample	Dilution		
	1/300	1/900	1/2700
untreated	++	+	-
70°C, 10 min	+	-	-
56°C, 30 min	+	-	-
90°C, 10 min	-	-	-

[++ means OCIF activity inhibiting osteoclast development more than 80%,
+means OCIF activity inhibiting osteoclast development between 30% and 80%,
and - means no OCIF activity.]

EXAMPLE 6

Internal amino acid sequence of OCIF protein

Each 2 fractions (1 ml) from No. 51-70 of blue-5PW fraction was acidified with 10 μ l of 25% TFA, and was applied to a reverse phase C4 column (BU-300, 2.1x220mm, manufactured by Perkin-Elmer Co.) equilibrated with 25% of acetonitrile containing 0.1 % TFA. The adsorbed protein was eluted with a 12 ml linear gradient of 25 to 55% acetonitrile at a flow rate of 0.2 ml/min, and the protein fractions corresponding to peak 6 and peak 7 were collected, respectively. The protein of each peak was applied to a protein sequencer (PROCISE 494, Perkin-Elmer Co.). However, the N-terminal sequence of the protein of each peak could not be analyzed. Therefore, N-terminal of the protein of each peak was considered to be blocked. So, internal amino acid sequences of these proteins were analyzed.

The protein of peak 6 or peak 7 purified by C4-HPLC was concentrated by centrifugation and pyridylethylated under reducing conditions. Briefly, 50 μ l of 0.5 M Tris-HCl, pH 8.5, containing 100 μ g of dithiothreitol, 10mM EDTA, 7 M guanidine-HCl, and 1% CHAPS was added to each samples, and the mixture was incubated overnight in the dark at a room temperature. Each the mixture was acidified with 25% TFA (a final concentration 0.1%) and was applied to a reversed phase C4 column (BU-300, 2.1x30mm, Perkin-Elmer Co.) equilibrated with 20 % acetonitrile containing 0.1 % TFA. The pyridil-ethylated OCIF

protein was eluted with a 9 ml linear gradient from 20 to 50% acetonitrile at a flow rate of 0.3 ml/min, and each protein peak was collected. The pyridil-ethyrate OCIF protein was concentrated under vacuum, and dissolved in 25 μ l of 0.1 M Tris-HCl, pH 9, containing 8 M Urea, and 0.1 % Tween 80. Seventy three μ l of 0.1 M Tris-HCl, pH 9, and 0.02 μ g of lysyl endopeptidase (Wako Pure Chemical, Japan) were added to the tube, and incubated at 37 °C for 15 hours. Each digest was acidified with 1 μ l of 25% TFA and was applied to a reverse phase C8 column (RP-300, 2.1x220mm, Perkin-Elmer Co.) equilibrated with 0.1% TFA.

The peptide fragments were eluted from the column with linear gradient from 0 to 50 % acetonitrile at a flow rate of 0.2 ml/min for 70 min., and each peptide peak was collected. Each peptide fragment (P1 - P3) was applied to the protein sequencer. The sequences of the peptides were shown in Sequence Numbers 1 - 3, respectively.

EXAMPLE 7

Determination of nucleotide sequence of the OCIF cDNA

i) Isolation of poly(A) + RNA from IMR-90 cells

About 10 ug of poly(A) + RNA was isolated from 1×10^8 cells of IMR-90 by using Fast Track mRNA isolation kit (Invitrogen) according to the manufacturer's instructions.

ii) Preparation of mixed primers

The following two mixed primers were synthesized based on the amino acid

sequences of two peptides (peptide P2 and peptide P3, sequence numbers 2 and 3, respectively). All the oligonucleotides in the mixed primers No. 2F can code for the amino acid sequence from the sixth residue, glutamine (Gln) to the twelfth residue, leucine (Leu), in peptide P2. All the oligonucleotides in the mixed primers No. 3R can code for the amino acid sequence from the sixth residue, histidine (His), to the twelfth residue, lysine (Lys), in peptide P3. The sequences of the mixed primers No. 2F and No. 3R were shown in Table 3.

Table 3

No. 2F

5' -CAAGAACAAA CTTTCAATT-3'
 G G G C C GC
 A
 G

No. 3R

5' -TTTATACATT GTAAAAGAAT G-3'
 C G C G GCTG
 A C
 G T

iii) Amplification of OCIF cDNA fragment by PCR (Polymerase chain reaction)

First strand cDNA was generated using Superscript II cDNA synthesis kit

(Gibco BRL) and 1 ug of poly(A) + RNA obtained in the example 7-i) according to the manufacturer's instructions. The DNA fragment encoding OCIF was obtained by PCR using the cDNA template and the primers shown in EXAMPLE 7-ii).

PCR was performed with the conditions as follows;

10X Ex Taq Buffer (Takara Shuzo)	5	ul
2.5 mM solution of dNTPs	4	ul
cDNA solution	1	ul
Ex Taq (Takara Shuzo)	0.25	ul
sterile distilled water	29.75	ul
40 uM solution of primers No. 2F	5	ul
40 uM solution of primers No. 3R	5	ul

The components of the reaction were mixed in a microcentrifuge tube. An initial denaturation step at 95 °C for 3 min was followed by 30 cycles of denaturation at 95°C for 30 sec annealing at 50 °C for 30 sec and extension at 70 °C for 2min. After the amplification, final extension step was performed at 70 °C for 5min. The size of PCR products were determined on a 1.5 % agarose gel electrophoresis. About 400 bp OCIF DNA fragment was obtained.

EXAMPLE 8

Cloning of the OCIF cDNA fragment amplified by PCR and determination of its DNA sequence

The OCIF cDNA fragment amplified by PCR in EXAMPLE 7-iii) was inserted in the plasmid, pBluescript II SK⁻ using DNA ligation kit ver. 2 (Takara Shuzo) according to the method by Marchuk, D. et al. (Nucleic Acids Res., vol 19, p1154, 1991). E.coli. DH5 α (Gibco BRL) was transformed with ligation mixture. The transformants were grown and a plasmid containing the OCIF cDNA (about 400 bp) was purified using the commonly used method. This plasmid was called pBSOCIF. The sequence of OCIF cDNA in pBSOCIF was determined using Taq Dye Deoxy Terminator Cycle Sequencing kit (Perkin Elmer). The size of the OCIF cDNA is 397 bp. The OCIF cDNA encodes an amino acid sequence containing 132 residues. The amino acid sequences of the internal peptides (peptide P2 and peptide P3, sequence number 2 and 3, respectively) that were used to design the primers were found at N- or C- terminal side in the amino acid sequence of the 132 amino acid polypeptide predicted by the 397 bp OCIF cDNA. In addition, the amino acid sequence of the internal peptide P1 (sequence number 1) was also found in the predicted amino acid sequence of the polypeptide. These data show that the 397 bp OCIF cDNA is a portion of the full length OCIF cDNA.

EXAMPLE 9

Preparation of the DNA probe

The 397 bp OCIF cDNA was prepared according to the conditions described in EXAMPLE 7-iii). The OCIF cDNA was subjected to a preparative agarose gel electrophoresis. The OCIF cDNA was purified from the gel using QIAEX gel extraction kit (QIAGEN), labeled with [α ³²P]dCTP using Megaprime DNA labeling

system (Amersham) and used to select a phage containing the full length OCIF cDNA.

EXAMPLE 10

Preparation of the cDNA library

cDNA was generated using Great Lengths cDNA synthesis kit (Clontech), oligo (dT) primer, [$\alpha^{32}\text{P}$]dCTP and 2.5 ug of poly(A) + RNA obtained in the example 7-i) according to the manufacturer's instructions. EcoRI-SalI-NotI adaptor was ligated to the cDNA. The cDNA was separated from the free adaptor and unincorporated free [$\alpha^{32}\text{P}$]dCTP. The purified cDNA was precipitated with ethanol and dissolved in 10 ul of TE buffer (10 mMTris-HCl (pH8.0), 1 mM EDTA). The cDNA with the adaptor was inserted in λ ZAP EXPRESS vector (Stratagene) at EcoRI site. The recombinant λ ZAP EXPRESS phage DNA containing the cDNA was in vitro packaged using Gigapack gold II packaging extract (Stratagene) and recombinant λ ZAP EXPRESS phage library was prepared.

EXAMPLE 11

Screening of recombinant phage

Recombinant phages obtained in EXAMPLE 10 were infected to E. Coli, XL1-Blue MRF' (Stratagene) at 37 °C for 15 min.. The infected E.coli cells were added to NZY medium containing 0.7 % agar at 50°C and plated on the NZY agar plates. After the plates were incubated at 37 °C overnight, Hybond N (Amersham) were placed on the surface of plates containing plaques. The membranes were denatured in the alkali solution, neutralized, and washed in

2xSSC according to the standard protocol. The phage DNA was immobilized on the membranes using UV Crosslink (Stratagene). The membranes were incubated in the hybridization buffer (Amersham) containing 100 μ g/ml salmon sperm DNA at 65°C for 4 hours and then incubated at 65 °C overnight in the same buffer containing 2×10^5 cpm/ml denatured OCIF DNA probe. The membranes were washed twice with 2xSSC and twice with a solution containing 0.1xSSC and 0.1 % SDS at 65 °C for 10 min each time. The positive clones were purified by repeating the screening twice. The purified λ ZAP EXPRESS phage clone containing about 1.6 kb DNA insert was used in the experiments described below. This phage was called λ OCIF. The purified λ OCIF and the infected into E. Coli XL1-Blue MRF' (Stratagene) according to a protocol of λ ZAP EXPRESS cloning kit (Stratagene). The culture broth of infected XL1-Blue MRF' was prepared. Purified 10CIF and ExAssist helper phage (Stratagene) were co-infected into E. coli strain XL-1 blue MRF' according to the protocol supplied with the kit. The culture broth of the co-infected XL-1 blue MRF' was added to a culture of E. coli strain XLOR (Stratagene) to transform them. Thus we obtained a Kanamycin-resistant transformant harboring a plasmid designated pBKOCIF which is a pBKCMV (Stratagene) vector containing the 1.6 kb insert fragment. The transformant including the plasmid containing about 1.6 kb OCIF cDNA was obtained by picking up the kanamycin-resistant colonies. The plasmid was called pBKOCIF. The transformant has been deposited to National Institute of Bioscience and Human-Technology (NIBH), Agency of Industrial Science and Tecnology as "FERM BP-5267" as pBK/01F10. A national deposit (Accession number, FERM P-14998) was transfered to the international deposit, on October 25, 1995

according to the Budapest treaty. The transformant pBK/01F10 was grown and the plasmid pBKOCIF was purified according to the standard protocol.

EXAMPLE 12

Determination of the nucleotide sequence of OCIF cDNA containing the full coding region.

The nucleotide sequence of OCIF cDNA obtained in EXAMPLE 11 was determined using Taq Dye Deoxy Terminator Cycle Sequencing kit (Perkin Elmer). The primers used were T3, T7 primers (Stratagene) and synthetic primers designed according to the OCIF cDNA sequence. The sequences of these primers are shown in sequence numbers 16 to 29. The nucleotide sequence of the OCIF cDNA is shown in sequence number 6 and the amino acid sequence predicted by the cDNA sequence is shown in sequence number 5.

EXAMPLE 13

Production of recombinant OCIF by 293/EBNA cells

i) Construction of the plasmid for expressing OCIF cDNA

pBKOCIF containing about 1.6 kb OCIF cDNA was prepared as described in EXAMPLE 11, and digested with restriction enzymes, BamHI and XhoI. The OCIF cDNA insert was cut out, separated by an agarose gel electrophoresis, and purified using QIAEX gel extraction kit (QIAGEN). The purified OCIF cDNA insert was ligated using DNA ligation kit ver. 2 (Takara Shuzo) to the expression vector pCEP4 (Invitrogen) digested with restriction enzymes, BamHI and XhoI. E.coli. DH5 α (Gibco BRL) was transformed with the ligation mixture.

The transformants were grown and the plasmid containing the OCIF cDNA (about 1.6 kb) was purified using QIAGEN column (QIAGEN). The expression plasmid pCEPOCIF was precipitated with ethanol, and dissolved in sterile distilled water was used in the experiments described below.

ii) Transient expression of OCIF cDNA and analysis of the biological activity

Recombinant OCIF was produced using the expression plasmid, pCEPOCIF prepared in EXAMPLE 13-i) according to the method described below. 8×10^5 cells of 293/EBNA (Invitrogen) were inoculated in each well of the 6-well plate using IMDM containing 10 % fetal calf serum (Gibco BRL). After the cells were incubated for 24 hours, the culture medium was removed and the cells were washed with serum free IMDM. The expression plasmid, pCEPOCIF and lipofectamine (Gibco BRL) were diluted with OPTI-MEM (Gibco BRL) and were mixed, and added to the cells in each well according to the manufacture's instructions. Three μ g of pCEPOCIF and 12 μ l of lipofectamine were used for each transfection. After the cells were incubated with pCEPOCIF and lipofectamine for 38 hours, the medium was replaced with 1 ml of OPTI-MEM. After the transfected cells were incubated for 30 hours, the conditioned medium was harvested and used for the biological assay. The biological activity of OCIF was analysed according to the method described below. Bone marrow cells obtained from mice, 17 days-old, were suspended in α -MEM (manufactured by GIBCO BRL Co.) containing 10% FBS, 2×10^{-8} M activated vitamin D_3 , and each test sample, and were inoculated and cultured for 7 days at 37°C in humidified 5%CO₂ as described in EXAMPLE 2. During incubation, 160

μ l of old medium in each well was replaced with the same volume of the fresh medium containing test sample diluted with 1×10^{-8} M of activated vitamin D₃ and α -MEM containing FBS on day 3 and day 5. On day 7, after washing the wells with phosphate buffered saline, cells were fixed with ethanol/acetone (1:1) for 1 min. and then osteoclast development was tested using acid phosphatase activity measuring kit (Acid Phosphatase, Leucocyte, Catalog No.387-A, Sigma Co.). The decrease of the number of TRAP positive cells was taken as an OCIF activity. As result, the conditioned medium showed the same OCIF activity as natural OCIF protein from IMR-90 conditioned medium (Table 4).

Table 4

OCIF activity of 293/EBNA conditioned medium.

Cultured Cell	Dilution						
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
OCIF expression							
vector transfected	++	++	++	++	++	+	-
vector transfected	-	-	-	-	-	-	-
untreated	-	-	-	-	-	-	-

[++ ; OCIF activity inhibiting osteoclast development more than 80%, + ; OCIF activity inhibiting osteoclast development between 30% and 80%, and - ; no

OCIF activity.]

iii) Isolation of recombinant OCIF protein from 293/EBNA-conditioned medium
293/EBNA-conditioned medium (1.8 l) obtained by cultivating the cells described in example 13-ii) was supplemented with 0.1 % of CHAPS and filtrated with 0.22 μ m membrane filter (Steribecs GS, Milipore Co.). The conditioned medium was applied to 50 ml of a heparin Sepharose CL-6B column (2.6 x 10 cm, Pharmacia Co.) equilibrated with 10mM Tris-HCl, pH 7.5. After washing the column with 10mM Tris-HCl, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 2 M NaCl at a flow rate of 4 ml/min for 100 min. and fractions (8 ml) were collected. Using 150 μ l of each fraction, OCIF activity was assayed according to the method described in EXAMPLE 2. OCIF active fraction (112 ml) eluted with approximately 0.6 to 1.2 M NaCl was obtained.

One hundred twelve ml of the active fraction was diluted to 1000 ml with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5, and applied to a heparin affinity column (heparin-5PW, 0.8 x 7.5 cm, Tosoh Co.) equilibrated with 10mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 10mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 2 M NaCl at a flow rate of 0.5ml/min for 60 min., and fractions (0.5 ml) were collected. Four μ l of each fraction was analyzed by SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions as described in EXAMPLE 4. On SDS-PAGE under reducing conditions, a single band of rOCIF protein with an apparent 60 KD was detected in fractions from 30 to 32, under

non-reducing conditions, bands of rOCIF protein with an apparent 60 KD and 120 KD were also detected in fractions from 30 to 32. The isolated rOCIF fraction from 30 to 32 was designated as recombinant OCIF derived from 293/EBNA (rOCIF(E)). 1.5 ml of the rOCIF(E) (535 μ g/ml) was obtained when determined by the method of Lowry using bovine serum albumin as a standard protein.

EXAMPLE 14

Production of recombinant OCIF using CHO cells

i) Construction of the plasmid for expressing OCIF

pBKOCIF containing about 1.6 kb OCIF cDNA was prepared as described in EXAMPLE 11, and digested with restriction enzymes, SalI and EcoRV. About 1.4 kb OCIF cDNA insert was separated by an agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The expression vector, pcDL-SR α 296 (Molecular and Cellular Biology, vol 8, p466, 1988) was digested with restriction enzymes, PstI and KpnI. About 3.4 kb of the expression vector fragment was cut out, separated by agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The ends of the purified OCIF cDNA insert and the expression vector fragment were blunted using DNA blunting kit (Takara Shuzo). The purified OCIF cDNA insert and the expression vector fragment were ligated using DNA ligation kit ver. 2 (Takara Shuzo). E.coli. DH5a α (Gibco BRL) was transformed with the ligation mixture. The transformant containing the OCIF expression plasmid, pSR α OCIF was obtained.

ii) Preparation of expression plasmid

The transformant containing the OCIF expression plasmid, pSR α OCIF prepared in the example 13-i) and the transformant containing the mouse DHFR expression plasmid, pBAdDSV shown in WO92/01053 were grown according to the standard method. Both plasmids were purified by alkali treatment, polyethylene glycol precipitation, and cesium chrolide density gradient ultra centrifugation according to method of Maniatis et al. (Molecular cloning, 2nd edition).

iii) Adaptation of CHOdhFr- cells to the protein free medium

CHOdhFr- cells (ATCC, CRL 9096) were cultured in IMDM containing 10 % fetal calf serum. The cells were adapted to EX-CELL 301 (JRH Bioscience) and then adapted to EX-CELL PF CHO (JRH Bioscience) according to the manufacture's instructions.

iv) Transfection of the OCIF expression plasmid, and the mouse DHFR expression plasmid, to CHOdhFr- cells.

CHOdhFr- cells prepared in EXAMPLE 14-iii) were transfected by electroporation with pSR α OCIF and pBAdDSV prepared in EXAMPLE 14-ii). 200 μ g of pSR α OCIF and 20 μ g of pBAdDSV were dissolved under sterile conditions in 0.8 ml of IMDM (Gibco BRL) containing 10 % fetal calf serum CG. 2×10^7 cells of CHOdhFr- were suspended in 0.8 ml of this medium. The cell suspension was transfered to a cuvette (Bio Rad) and the cells were transfected by electroporation using gene pulser (Bio Rad) under condition of

360 V and 960 μ F. The suspension of electroporated cells was transferred to T-flasks (Sumitomo Bakelite) containing 10 ml of EX-CELL PF-CHO, and incubated in the CO₂ incubator for 2 days. Then the transfected cells were inoculated in each well of a 96 well plate (Sumitomo Bakelite) at a density of 5000 cells/well and cultured for about 2 weeks. The transformants expressing DHFR are selected since EX-CELL PF-CHO does not contain nucleotides and the parental cell line CHO dhFr⁻ can not grow in this medium. Most of the transformants expressing DHFR express OCIF since the OCIF expression plasmid was used ten times as much as the mouse DHFR expression plasmid. The transformants whose conditioned medium had high OCIF activity were selected among the transformants expressing DHFR according to the method described in EXAMPLE 2. The transformants that express large amounts of OCIF were cloned by limiting dilution. The clones whose conditioned medium had high OCIF activity were selected as described above and the transformant expressing large amount of OCIF, 5561, was obtained.

v) Production of recombinant OCIF

To produce recombinant OCIF (rOCIF), EX-CELL 301 medium (3 l) in a 3 l-spiner flask was inoculated with the clone (5561) at a cell-density of 1×10^5 cells/ml. The 5561 cells were cultured in a spiner flask at 37°C for 4 to 5 days. When the concentration of the 5561 cells reached to 1×10^6 cells/ml, about 2.7 l of the conditioned medium was harvested. Then about 2.7 l of EX-CELL 301 was added to the spiner flask and the 5561 cells were cultured repeatedly. About 20 l of the conditioned medium was harvested using the three spiner

flasks.

vi) Isolation of recombinant OCIF protein from CHO cells-conditioned medium

CHO cells-conditioned medium (1.0 l) described in EXAMPL 14-v) was supplemented with 1.0 g of CHAPS and filtrated with 0.22 μ m membrane filter (Steribecks GS, Milipore Co.). The conditioned medium was applied to a heparin Sepharose-FF column (2.6 x 10 cm, Pharmacia Co.) equilibrated with 10 mM Tris-HCl, pH 7.5. After washing the column with 10 mM Tris-HCl, 0.1 % CHAPS, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 2 M NaCl at a flow rate of 4 ml/min for 100 min. and fractions (8 ml) were collected. Using 150 μ l of each fraction, OCIF activity was assayed according to the method described in EXAMPLE 2. Active fraction (112 ml) eluted with approximately 0.6 to 1.2 M NaCl was obtained.

The 112 ml of active fraction was diluted to 1200 ml with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5, and applied to a affinity column (blue-5PW, 0.5 x 5.0 cm, Tosoh Co.) equilibrated with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 3 M NaCl at a flow rate of 0.5ml/min for 60 min., and fractions (0.5 ml) were collected. Four μ l of each fraction was subjected to SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions as described in EXAMPLE 4. On SDS-PAGE under reducing conditions, a single band of rOCIF protein with apparent 60 KD was detected in fractions 30 to 38, under non-reducing conditions, bands of rOCIF protein with apparent 60 KD and 120 KD were also detected in fractions

30 to 38. The isolated rOCIF fraction, 30 to 38, was designated as purified recombinant OCIF derived from CHO cells (rOCIF(C)). 4.5 ml of the rOCIF(C) (113 μ g/ml) was obtained when determined by the method of Lowry using bovine serum albumin as a standard protein.

EXAMPLE 15

Determination of N-terminal amino acid sequence of rOCIFs

Each 3 μ g of the isolated rOCIF(E) and rOCIF(C) was adsorbed to polyvinylidene difluoride (PVDF) membranes with Prospin (PERKIN ELMER Co.). The membranes were washed with 20 % ethanol and the N-terminal amino acid sequences of the adsorbed proteins were analyzed by protein sequencer (PROCISE 492, PERKIN ELMER Co.). The determined N-terminal amino acid sequence is shown in sequence No. 7.

The N-terminal amino acid of rOCIF(E) and rOCIF(C) was the 22th amino acid of glutamine from Met as translation starting point, as shown in sequence number 5. The 21 amino acids from Met to Gln were identified as a signal peptide. The N-terminal amino acid sequence of OCIF isolated from IMR-90 conditioned medium was undetectable. Accordingly, the N-terminal glutamine of OCIF may be blocked by converting from glutamine to pyroglutamine within culturing or purifying.

EXAMPLE 16

Biological activity of recombinant(r) OCIF and natural(n) OCIF

i) Inhibition of vitamin D₃ induced osteoclast formation from murine bone marrow cells

Each the rOCIF(E) and nOCIF sample was diluted with α -MEM (GIBCO BRL Co.) containing 10% FBS and 2×10^{-8} M of activated vitamin D₃ (a final concentration of 250 ng/ml). Each sample was serially diluted with the same medium, and 100 μ l of each diluted sample was added to each well in 96-well

plates. Bone marrow cells obtained from mice, 17 days-old, were inoculated at a cell density of 3×10^5 cells/ $100 \mu\text{l}$ / well to each well in 96-well plates and cultured for 7 days at 37°C in humidified $5\%\text{CO}_2$. On day 7, the cells were fixed and stained with a acid phosphatase measuring kit (Acid Phosphatase, Leucocyte, No387-A, Sigma) according to the method described in EXAMPLE 2. The decrease of acid phosphatase activity (TRAP) was taken as OCIF activity. The decrease of acid phosphatase-positive cells was evaluated by solubilizing the pigment of dye and measuring absorbance. In detail, $100 \mu\text{l}$ of a mixture of 0.1 N NaOH and dimethylsulfoxide (1:1) was added to each well and the well was vibrated to solubilize the dye. After solubilizing the dye completely, an absorbance of each well was measured at 590 nm subtracting the absorbance at 490 nm using microplate reader (Immunoreader NJ-2000, InterMed). The microplate reader was adjusted to 0 absorbance using a well with monolayered bone marrow cells which was cultured in the medium without activated vitamin D_3 . The decrease of TRAP activity was expressed as a percentage of the control absorbance value (=100%) of the solubilized dye from wells with bone marrow cells which were cultured in the absence of OCIF. The results are shown in Table 5.

Table 5

Inhibition of vitamin D_3 -induced osteoclast formation from murine bone marrow cells

OCIF concentration(ng/ml)	250	125	63	31	16	0
rOCIF (E)	0	0	3	62	80	100

nOCIF.	0	0	27	27	75	100 (%)
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Both nOCIF and rOCIF(E) inhibited osteoclast formation in a dose dependent manner in the concentration of 16 ng/ml or higher

ii) Inhibition of vitamin D3-induced osteoclast formation in co-cultures of stromal cells and mouse spleen cells.

Effect of OCIF on osteoclast formation induced by Vitamin D₃ in co-cultures of stromal cells and mouse spleen cells was tested according to the method of N. Udagawa et al. (Endocrinology, vol. 125, p1805-1813, 1989). In detail, each of rOCIF(E), rOCIF(C), and nOCIF sample was serially diluted with α -MEM (GIBCO BRL Co.) containing 10% FBS, 2×10^{-8} M of activated vitamin D₃, and 2×10^{-7} M dexamethasone, and 100 μ l of each the diluted samples was added to each well in 96 well-microwell plates. Murine bone marrow-derived stromal ST2 cells (RIKEN Cell Bank RCB0224) ; 5×10^3 cells per 100 μ l of α -MEM containing 10% FBS, and spleen cells from ddy mice, 8 weeks-old, ; 1×10^5 cells per 100 μ l in the same medium, were inoculated to each well in 96-well plates and cultured for 5 days at 37°C in humidified 5%CO₂. On day 5, the cells were fixed and stained with a kit for acid phosphatase (Acid Phosphatase, Leucocyte, No387-A, Sigma). The decrease of acid phosphatase-positive cells was taken as OCIF activity. The decrease of acid phosphatase-positive cells was evaluated according to the method described in EXAMPLE 16-i). The results are shown in Table 6 ; rOCIF(E) and rOCIF(C), and Table 7 ; rOCIF(E) and nOCIF.

Table 6

Inhibition of osteoclast formation in co-cultures of stromal cells and mouse spleen cells.

OCIF concentration(ng/ml)	50	25	13	6	0
rOCIF(E)	3	22	83	80	100
rOCIF(C)	13	19	70	96	100 (%)

Table 7

Inhibition of osteoclast formation in co-cultures of stromal cells and mouse spleen cells.

OCIF concentration(ng/ml)	250	63	16	0
rOCIF(E)	7	27	37	100
rOCIF(C)	13	23	40	100 (%)

nOCIF, rOCIF(E) and rOCIF(C) inhibited osteoclast formation in a dose dependent manner in the concentration of 6 - 16 ng/ml or higher

iii) Inhibition of PTH-induced osteoclast formation from murine bone marrow cells.

Effect of OCIF on osteoclast formation induced by PTH was tested according to the method of N. Takahashi et al. (Endocrinology, vol. 122,

p1373-1382, 1988). In detail, each the rOCIF(E) and nOCIF sample (125 ng/ml) was serially diluted with α -MEM (manufactured by GIBCO BRL Co.) containing 10% FBS and 2×10^{-8} M PTH, and 100 μ l of each the diluted samples was added to 96 well-plates. Bone marrow cells from ddy mice, 17 days-old, at a cell density of 3×10^5 cells per 100 μ l of α -MEM containing 10% FBS were inoculated to each well in 96-wells plates and cultured for 5 days at 37°C in humidified 5%CO₂. On day 5, the cells were fixed with ethanol/acetone (1:1) for 1 min. at room temperature and stained with a kit for acid phosphatase (Acid Phosphatase, Leucocyte, No387-A, Sigma) according to the method described in EXAMPLE 2. The decrease of acid phosphatase-positive cells was taken as OCIF activity. The decrease of acid phosphatase-positive cells was evaluated according to the method described in EXAMPLE 16-i). The results are shown in Table 8.

Table 8

Inhibition of PTH-induced osteoclast formation from murine bone marrow cells.

OCIF concentration(ng/ml)	125	63	31	16	8	0
rOCIF(E)	6	58	58	53	88	100
nOCIF	18	47	53	56	91	100

nOCIF and rOCIF(E) inhibited osteoclast formation in a dose dependent manner in the concentration of 16 ng/ml or higher

iv) Inhibition of IL-11-induced osteoclast formation

Effect of OCIF on osteoclast formation induced by IL-11 was tested according to the method of T. Tamura et al. (Proc. Natl. Acad. Sci. USA, vol. 90, p11924-11928, 1993). In detail, each rOCIF(E) and nOCIF sample was serially diluted with α -MEM (GIBCO BRL Co.) containing 10% FBS and 20 ng/ml IL-11 and 100 μ l of each the diluted sample was added to each well in 96-well plates. Newborn mouse calvaria-derived pre-adipocyte MC3T3-G2/PA6 cells (RIKEN Cell Bank RCB1127) ; 5×10^3 cells per 100 μ l of α -MEM containing 10% FBS, and spleen cells from ddy mouse, 8 weeks-old, ; 1×10^5 cells per 100 μ l in the same medium, were inoculated to each well in 96-well plates and cultured for 5 days at 37 °C in humidified 5%CO₂. On day 5, the cells were fixed and stained with a kit for acid phosphatase (Acid Phosphatase, Leucocyte, No387-A, Sigma). Acid phosphatase positive cells were counted under microscope and a decrease of the cell numbers was taken as OCIF activity. The results are shown in Table 9.

Table 9

OCIF concentration(ng/ml)	500	125	31	7.8	2.0	0.5	0
nOCIF	0	0	1	4	13	49	31
rOCIF(E)	0	0	1	3	10	37	31

Both nOCIF and rOCIF(E) inhibited osteoclast formation in a dose dependent manner in the concentration of 2 ng/ml or higher

The results shown in Table 4-8 indicated that OCIF inhibits all the

vitamin D₃, PTH, and IL-11-induced osteoclast formations at almost the same doses. Accordingly, OCIF would be able to be used for treatment of the different types of bone disorders with decreased bone mass, which are caused by different substances which induce bone resorption.

EXAMPLE 17

Isolation of monomer-type OCIF and dimer-type OCIF

Each rOCIF(E) and rOCIF(C) sample containing 100 μ g of OCIF protein, was supplemented with 1/100 volume of 25 % trifluoro acetic acid and applied to a reverse phase column (PROTEIN-RP, 2.0x250 mm, YMC Co.) equilibrated with 30 % acetonitrile containing 0.1 % trifluoro acetic acid. OCIF protein was eluted from the column with linear gradient from 30 to 55 % acetonitrile at a flow rate of 0.2 ml/min for 50 min. and each OCIF peak was collected. Each the monomer-type OCIF peak fraction and dimer-type OCIF peak fraction was lyophilized, respectively.

EXAMPLE 18

Determination of molecular weight of recombinant OCIFs

Each 1 μ g of the isolated monomer-type and dimer-type nOCIF purified using reverse phase column according to EXAMPLE 3-iv) and each 1 μ g of monomer-type and dimer-type rOCIF described in EXAMPLE 17 was concentrated under vacuum, respectively. Each sample was incubated in the buffer for SDS-PAGE, subjected to SDS-polyacrylamide gel electrophoresis, and protein bands on the gel were stained with silver according to the method described in

EXAMPLE 4. Results of electrophoresis under non-reducing conditions and reducing conditions are shown in Figure 6 and Figure 7.

A protein band with an apparent molecular weight of 60 KD was detected in each monomer-type OCIF sample, and a protein band with an apparent molecular weight of 120 KD was detected in each dimer-type OCIF sample in non-reducing conditions. A protein band with an apparent molecular weight of 60 KD was detected in each monomer-type OCIF sample under reducing conditions. Accordingly, molecular weights of monomer-type nOCIF from IMR-90 cells, rOCIF from 293/EBNA cells and rOCIF from CHO cells were almost the same. Molecular weights of dimer-type nOCIF from IMR-90 cells, rOCIF from 293/EBNA cells, and rOCIF from CHO cells were also the same.

EXAMPLE 19

Remove N-linked Oligosaccharide chain and Mesuring molecular weight of natural and recombinant OCIF

Each sample containing 5 μ g of the isolated monomer-type and dimer-type nOCIF purified using reverse phase column according to EXAMPLE 3-iv) and each sample containing 5 μ g of monomer-type and dimer-type rOCIF described in EXAMPLE 17 were concentrated under vaccum. Each sample was dissolved in 9.5 μ l of 50 mM sodium phosphate buffer, pH 8.6, containing 100 mM 2-mercaptoethanol, supplemented with 0.5 μ l of 250 U/ml N-glycanase (Seikagaku kogyo Co.) and incubated for one day at 37 °C. Each sample was supplemented with 10 μ l of 20 mM Tris-HCl, pH 8.0 containing 2 mM EDTA, 5 % SDS, and 0.02 % bromo-phenol blue and heated for 5 min at 100 °C. Each 1 μ l

of the samples was subjected to SDS-polyacrylamide gel electrophoresis, and protein bands on the gel were stained with silver as described in EXAMPLE 4. The patterns of electrophoresis are shown in Figure 8.

An apparent molecular weight of each the deglycosylated nOCIF from IMR-90 cells, rOCIF from CHO cells, and rOCIF from 293/EBNA cells was 40 KD under reducing conditions. An apparent molecular weight of each untreated nOCIF from IMR-90 cells, rOCIF from 293/EBNA cells, and rOCIF from CHO cells was 60 KD under reducing conditions. Accordingly, the results indicate that the OCIF proteins are glycoproteins with N-linked sugar chains.

EXAMPLE 20

Cloning of OCIF variant cDNAs and determination of their DNA sequences

The plasmid pBKOCIF, which is inserted OCIF cDNA to pBKCMV (Stratagene), was obtained from one of some purified positive phage as in example 10 and 11. And more, during the screening of the cDNA library with the 397 bp OCIF cDNA probe, the transformants containing plasmids whose insert sizes were different from that of pBKOCIF were obtained. These transformants containing the plasmids were grown and the plasmids were purified according to the standard method. The sequence of the insert DNA in each plasmid was determined using Taq Dye Deoxy Terminator Cycle Sequencing kit (Perkin Elmer). The used primers were T3, T7 primers (Stratagene) and synthetic primers prepared based on the nucleotide sequence of OCIF cDNA. There are four OCIF variants (OCIF2, 3, 4, and 5) in addition to OCIF. The nucleotide sequence of OCIF2 is shown in the sequence number 8 and the amino acid sequence of OCIF 2 predicted by the nucleotide sequence is shown in the sequence number 9. The nucleotide sequence of OCIF3 is shown in the sequence number 10 and the amino acid sequence of OCIF3 predicted by the nucleotide sequence is shown in the sequence number 11. The nucleotide sequence of OCIF4 is shown in the sequence number 12 and the amino acid sequence of OCIF4 predicted by the nucleotide sequence is shown in the sequence number 13. The nucleotide sequence of OCIF5 is shown in the sequence number 14 and the amino acid sequence of OCIF5 predicted by the nucleotide sequence is shown in the sequence number 15. The structures of OCIF variants are shown in Figures 9 to 12 and are described in brief below.

OCIF2

OCIF2 cDNA has a deletion of 21 bp from guanine at nucleotide number 265 to guanine at nucleotide number 285 in OCIF cDNA (sequence number 6). Accordingly OCIF2 has a deletion of 7 amino acids from glutamic acid (Glu) at amino acid number 68 to glutamine (Gln) at amino acid number 74 in OCIF (sequence number 5).

OCIF3

OCIF3 cDNA has a point mutation at nucleotide number 9 in OCIF cDNA (sequence number 6) where cytidine is replaced with guanine. Accordingly OCIF3 has a mutation and asparagine (Asn) at amino acid number -19 in OCIF (sequence number 5) is replaced with lysine (Lys). The mutation seems to be located in the signal sequence and have no essential effect on the secreted OCIF3. OCIF3 cDNA has a deletion of 117 bp from guanine at nucleotide number 872 to cytidine at nucleotide number 988 in OCIF cDNA (sequence number 6).

Accordingly OCIF3 has a deletion of 39 amino acids from threonine (Thr) at amino acid number 270 to leucine (Leu) at amino acid number 308 in OCIF (sequence number 5).

OCIF4

OCIF4 cDNA has two point mutations in OCIF cDNA (sequence number 6). Cytidine at nucleotide number 9 is replaced with guanine and guanine at nucleotide number 22 is replaced with thymidine in OCIF cDNA (sequence number 6).

Accordingly OCIF4 has two mutations. Asparagine (Asn) at amino acid number -19 in OCIF (sequence number 5) is replaced with lysine (Lys), and alanine (Ala)

at amino acid number -14 is replaced with serine (Ser). These mutations seem to be located in the signal sequence and have no essential effect on the secreted OCIF4.

OCIF4 cDNA has about 4 kb DNA, which is the intron 2 of OCIF gene, inserted between nucleotide number 400 and nucleotide number 401 in OCIF cDNA (sequence number 6). The open reading frame stops in intron 2.

Accordingly OCIF4 has an additional novel amino acid sequence containing 21 amino acids after alanine (Ala) at amino acid number 112 in OCIF (sequence number 5).

OCIF5

OCIF5 cDNA has a point mutation at nucleotide number 9 in OCIF cDNA (sequence number 6) where cytidine is replaced with guanine.

Accordingly OCIF5 has a mutation and asparagine (Asn) at amino acid number -19 in OCIF (sequence number 5) is replaced with lysine (Lys). The mutation seems to be located in the signal sequence and have no essential effect on the secreted OCIF5.

OCIF5 cDNA has the latter portion (about 1.8 kb) of intron 2 between nucleotide number 400 and nucleotide number 401 in OCIF cDNA (sequence number 6). The open reading frame stops in the latter portion of intron 2.

Accordingly OCIF5 has an additional novel amino acid sequence containing 12 amino acids after alanine (Ala) at amino acid number 112 in OCIF (sequence number 5).

EXAMPLE 21

Production of OCIF variants

i) Construction of the plasmid for expressing OCIF variants

The plasmid containing OCIF2 or OCIF3 cDNA was obtained as described in EXAMPLE 20 and called pBKOCIF2 and pBKOCIF3, respectively. pBKOCIF2 and pBKOCIF3 were digested with restriction enzymes, BamHI and XhoI. The OCIF2 and OCIF3 cDNA inserts were separated by agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The purified OCIF2 and OCIF3 cDNA inserts were individually ligated using DNA ligation kit ver. 2 (Takara Shuzo) to the expression vector pCEP4 (Invitrogen) that had been digested with restriction enzymes, BamHI and XhoI. E.coli. DH5 α (Gibco BRL) was transformed with the ligation mixture.

The plasmid containing OCIF4 cDNA was obtained as described in EXAMPLE 20 and called pBKOCIF4. pBKOCIF4 was digested with restriction enzymes, SpeI and XhoI (Takara Shuzo). The OCIF4 cDNA insert was separated by an agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The purified OCIF4 cDNA insert was ligated using DNA ligation kit ver. 2 (Takara Shuzo) to the expression vector pCEP4 (Invitrogen) that had been digested with restriction enzymes, NheI and XhoI (Takara Shuzo). E.coli. DH5 α (Gibco BRL) was transformed with the ligation mixture.

The plasmid containing OCIF5 cDNA was obtained as described in EXAMPLE 20 and was called pBKOCIF5. pBKOCIF5 was digested with restriction enzyme, HindIII (Takara Shuzo). The 5' portion of the coding region in the OCIF5 cDNA insert was separated by agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The OCIF expression plasmid, pCEPOCIF,

obtained in EXAMPLE 13-i) was digested with restriction enzyme, HindIII (Takara Shuzo). The 5' portion of the coding region in the OCIF cDNA was removed. The rest of the plasmid that contains pCEP vector and the 3' portion of the coding region of OCIF cDNA was called pCEPOCIF-3'. pCEPOCIF-3' was separated by an agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The OCIF5 cDNA HindIII fragment and pCEPOCIF-3' were ligated using DNA ligation kit ver. 2 (Takara Shuzo). E.coli. DH5 α (Gibco BRL) was transformed with the ligation mixture.

The obtained transformants were grown at 37 °C overnight and the OCIF variants expression plasmids (pCEPOCIF2, pCEPOCIF3, pCEPOCIF4, and pCEPOCIF5) were purified using QIAGEN column (QIAGEN). These OCIF-variants-expression plasmids were precipitated with ethanol, dissolved in sterile distilled water, and used in the experiments described below.

ii) Transient expression of OCIF variant cDNAs and analysis of the biological activity of recombinant OCIF variants.

Recombinant OCIF variants were produced using the expression plasmid, pCEPOCIF2, pCEPOCIF3, pCEPOCIF4, and pCEPOCIF5 prepared as described in EXAMPLE 21-i) according to the method described in EXAMPLE 13-ii). The biological activities of recombinant OCIF variants were analysed. The results were that these OCIF variants (OCIF2, OCIF3, OCIF4, and OCIF5) had a weak activity.

EXAMPLE 22

Preparation of OCIF mutants

i) Construction of a plasmid vector for subcloning cDNAs encoding OCIF mutants

The plasmid vector (5 μ g) described in EXAMPLE 11 was digested with restriction enzymes Bam HI and Xho I (Takara Shuzo). The digested DNA was subjected to a preparative agarose gel electrophoresis. DNA fragment with an approximate size of 1.6 kilobase pairs (kb) that contained the entire coding sequence for OCIF was purified from the gel using QIAEX gel extraction kit (QIAGEN). The purified DNA was dissolved in 20 μ l of sterile distilled water. This solution was designated DNA solution 1. p Bluescript II SK + (3 μ g) (Stratagene) was digested with restriction enzymes Bam HI and Xho I (Takara Shuzo). The digested DNA was subjected to preparative agarose gel electrophoresis. DNA fragment with an approximate size of 3.0 kb was purified from the gel using QIAEX DNA extraction kit (QIAGEN). The purified DNA was dissolved in 20 μ l of sterile distilled water. The solution was designated DNA solution 2. One microliter of DNA solution 2, 4 μ l of DNA solution 1 and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 (Takara Shuzo) were mixed and incubated at 16 °C for 30 min. (The ligation mixture was used for the transformation of E. coli in a manner described below). Conditions for transformation of E. coli were as follows. One hundred microliters of competent E. coli DH5 α cells (GIBCO BRL) and 5 μ l of the ligation mixture was mixed in a sterile 15-ml tube (IWAKI glass). The tube was kept on ice for 30 min. After incubation for 45 sec at 42°C, to the cells was added 250 μ l of L broth (1% Tryptone, 0.5% yeast extract, 1% NaCl). The cell suspension was then incubated for 1hr. at 37°C with shaking. Fifty

microliters of the cell suspension was plated onto an L-agar plate containing 50 μ g/ml of ampicillin. The plate was incubated overnight at 37°C.

Six colonies which grew on the plate were individually incubated in 2 ml each of L-broth containing 50 μ g/ml of ampicillin overnight at 37°C with shaking. The structure of the plasmids in the colonies was analyzed. A plasmid in which the 1.6-kb DNA fragment containing the entire OCIF cDNA is inserted between the digestion sites of Bam HI and Xho I of pBluescript II SK⁺ was obtained and designated as pSK⁺ -OCIF.

ii) Preparation of mutants in which one of the Cys residues in OCIF is replaced with Ser residue

1) Introduction of mutations into OCIF cDNA

OCIF mutants were prepared in which one of the five Cys residues present in OCIF at positions 174, 181, 256, 298 and 379 (in SEQUENCE NO 4) was replaced with Ser residue and were designated OCIF-C19S(174Cys to Ser), OCIF-C20S (181Cys to Ser), OCIF-C21S (256Cys to Ser), OCIF-C22S (298Cys to Ser) and OCIF-C23S (379Cys to Ser), respectively.

To prepare the mutants, nucleotides encoding the corresponding Cys residues were replaced with those encoding Ser. Mutagenesis was carried out by a two-step polymerase chain reaction (PCR). The first step of the PCRs consisted of two reactions, PCR 1 and PCR 2.

PCR 1	10X Ex Taq Buffer (Takara Shuzo)	10	μ l
	2.5 mM solution of dNTPs	8	μ l
	the plasmid vector described in EXAMPLE 11 (8ng/ml)	2	μ l
	sterile distilled water	73.5	μ l

	20 μ M solution of primer 1	5 μ l
	100 μ M solution of primer 2 (for mutagenesis)	1 μ l
	Ex Taq (Takara Shuzo)	0.5 μ l
PCR 2	10X Ex Taq Buffer (Takara Shuzo)	10 μ l
	2.5 mM solution of dNTPs	8 μ l
	the plasmid vector described in EXAMPLE 11 (8ng/ml)	2 μ l
	sterile distilled water	73.5 μ l
	20 μ M solution of primer 3	5 μ l
	100 μ M solution of primer 4 (for mutagenesis)	1 μ l
	Ex Taq (Takara Shuzo)	0.5 μ l

Specific sets of primers were used for each mutation and other components were unchanged. Primers used for the reactions are shown in Table 10. The nucleotide sequences of the primers are shown in SEQUENCE NO: 20,23,27 and 30-40. The PCRs were performed under the following conditions as follows. An initial denaturation step at 97°C for 3 min was followed by 25 cycles of denaturation at 95°C for 1 min annealing at 55°C for 1 min and extension at 72°C for 3 min. After these amplification cycles, final extension was performed at 70°C for 5 min. The size of the PCR products was confirmed by agarose gel electrophoresis using reaction solution. After the first PCR, excess primers were removed using Amicon microcon (Amicon). The final volume of the solutions that contained the PCR products were made to 50 μ l with sterile distilled water. These purified PCR products were used for the second PCR (PCR 3).

PCR 3	10X Ex Taq Buffer (Takara Shuzo)	10 μ l
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2.5 mM solution of dNTPs	8 μ l
solution containing DNA fragment obtained from PCR 1	5 μ l
solution containing DNA fragment obtained from PCR 2	5 μ l
sterile distilled water	61.5 μ l
20 μ M solution of primer 1	5 μ l
20 μ M solution of primer 3	5 μ l
Ex Taq (Takara Shuzo)	0.5 μ l

Table 10

mutants	primer-1	primer-2	primer-3	primer-4
OCIF-C19S	IF 10	C19SR	IF 3	C19SF
OCIF-C20S	IF 10	C20SR	IF 3	C20SF
OCIF-C21S	IF 10	C21SR	IF 3	C21SF
OCIF-C22S	IF 10	C22SR	IF 14	C22SF
OCIF-C23S	IF 6	C23SR	IF 14	C23SF

The reaction conditions were exactly the same as those for PCR 1 or PCR 2. The size of the PCR products was confirmed by 1.0 % or 1.5 % agarose gel electrophoresis. The DNA fragments were precipitated with ethanol, dried under vacuum and dissolved in 40 μ l of sterile distilled water. The solutions containing DNA fragments with mutation C19S, C20S, C21S, C22S and C23S were

designated as DNA solution A, DNA solution B, DNA solution C, DNA solution D and DNA solution E, respectively.

The DNA fragment which is contained in solution A (20 μ l) was digested with restriction enzymes Nde I and Sph I (Takara Shuzo). A DNA fragment with an approximate size of 400 base pairs (bp) was extracted from a preparative agarose gel and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated DNA solution 3. Two micrograms of pSK + -OCIF was digested with restriction enzymes Nde I and Sph I. A DNA fragment with an approximate size of 4.2 kb was purified from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as DNA solution 4. Two microliters of DNA solution 3, 3 μ l of DNA solution 4 and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent E. coli DH5 α cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C19S.

The DNA fragment which is contained in solution B (20 μ l) was digested with restriction enzymes Nde I and Sph I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated DNA solution 5. Two microliters of DNA solution 5, 3 μ l of DNA solution 4 and 5 μ l of ligation buffer I of DNA ligation kit

ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5 α cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C20S.

The DNA fragment which is contained in solution C (20 μ l) was digested with restriction enzymes Nde I and Sph I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as DNA solution 6. Two microliters of DNA solution 6, 3 μ l of DNA solution 4 and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5 α cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C21S.

The DNA fragment which is contained in solution D (20 μ l) was digested with restriction enzymes Nde I and Bst PI. A DNA fragment with an approximate size of 600 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as DNA solution 7. Two micrograms of pSK + -OCIF was digested with restriction enzymes Nde I and Bst PI. A DNA fragment with an approximate size of 4.0 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled

water. This DNA solution was designated as DNA solution 8. Two microliters of DNA solution 7, 3 μ l of DNA solution 8 and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5 α cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA in which the 600-bp Nde I-BstPI fragment with the mutation (the C22S mutation) is substituted for the 600-bp Nde I-Bst PI fragment of pSK+ -OCIF by analyzing the DNA structure. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C22S.

The DNA fragment which is contained in solution E (20 μ l) was digested with restriction enzymes Bst PI and Eco RV. A DNA fragment with an approximate size of 120 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as DNA solution 9. Two micrograms of pSK + -OCIF was digested with restriction enzymes Bst EII and Eco RV. A DNA fragment with an approximate size of 4.5 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as DNA solution 10. Two microliters of DNA solution 9, 3 μ l of DNA solution 10 and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation was carried out. Competent *E. coli* DH5 α cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by

DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C23S.

2) Construction of vectors for expressing the OCIF mutants

pSK-OCIF-C19S, pSK-OCIF-C20S, pSK-OCIF-C21S, pSK-OCIF-C22S and pSK-OCIF-C23S were digested with restriction enzymes Bam HI and Xho I. The 1.6 kb Bam HI-Xho I DNA fragment encoding each OCIF mutant was isolated and dissolved in 20 μ l of sterile distilled water. The DNA solutions that contain 1.6 kb cDNA fragments derived from pSK-OCIF-C19S, pSK-OCIF-C20S, pSK-OCIF-C21S, pSK-OCIF-C22S and pSK-OCIF-C23S were designated C19S DNA solution, C20S DNA solution, C21S DNA solution, C22S DNA solution and C23S DNA solution, respectively. Five micrograms of an expression vector pCEP 4 (Invitrogen) was digested with restriction enzymes Bam HI and Xho I. A DNA fragment with an approximate size of 10 kb was purified and dissolved in 40 μ l of sterile distilled water. This DNA solution was designated as pCEP 4 DNA solution. One microliter of pCEP 4 DNA solution and 6 μ l of either C19S DNA solution, C20S DNA solution, C21S DNA solution, C22S DNA solution or C23S DNA solution were independently mixed with 7 μ l of ligation buffer I of DNA ligation kit ver. 2 and ligation reactions were carried out. Competent *E. coli* DH5 α cells (100 μ l) were transformed with 7 μ l of each ligation mixture. Ampicillin-resistant transformants were screened for clones containing plasmid in which a 1.6-kb cDNA fragment is inserted between the recognition sites of Bam HI and Xho I of pCEP 4 by analyzing the DNA structure. The plasmids which were obtained containing the cDNA encoding OCIF-C19S, OCIF-C20S, OCIF-C21S, OCIF-C22S and OCIF-C23S were designated pCEP4-OCIF-C19S, pCEP4-OCIF-C20S, pCEP4-OCIF-C21S, pCEP4-OCIF-C22S and

pCEP4-OCIF-C23S, respectively.

ii) Preparation of domain-deletion mutants of OCIF

(1) deletion mutagenesis of OCIF cDNA

A series of OCIF mutants with deletions of from Thr 2 to Ala 42, from Pro 43 to Cys 84, from Glu 85 to Lys 122, from Arg 123 to Cys 164, from Asp 177 to Gln 251 and from Ile 252 to His 326 were prepared (positions of the amino acid residues are shown in SEQUENCE NO: 4). These mutants were designated as OCIF-DCR1, OCIF-DCR2, OCIF-DCR3, OCIF-DCR4, OCIF-DDD1 and OCIF-DDD2, respectively.

Mutagenesis was performed by two-step PCR as described in EXAMPLE 22-(ii). The primer sets for the reactions are shown in Table 11 and the nucleotide sequences of the primers are shown in SEQUENCE NO:19, 25, 40-53, and 54.

Table 11

mutants	primer-1	primer-2	primer-3	primer-4
OCIF-DCR1	XhoI F	DCR1R	IF 2	DCR1F
OCIF-DCR2	XhoI F	DCR2R	IF 2	DCR2F
OCIF-DCR3	XhoI F	DCR3R	IF 2	DCR3F
OCIF-DCR4	XhoI F	DCR4R	IF 16	DCR4F
OCIF-DDD1	IF 8	DDD1R	IF 14	DDD1F
OCIF-DDD2	IF 8	DDD2R	IF 14	DDD2F

The final PCR products were precipitated with ethanol, dried under vacuum and dissolved in 40 μ l of sterile distilled water. Solutions of DNA fragment coding for portions of OCIF-DCR1, OCIF-DCR2, OCIF-DCR3, OCIF-DCR4, OCIF-DDD1 and OCIF-DDD2 were designated as DNA solutions F, G, H, I, J and K, respectively.

The DNA fragment which is contained in solution F (20 μ l) was digested with restriction enzymes Nde I and Xho I. A DNA fragment with an approximate size of 500 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated DNA solution 11. Two micrograms of pSK+ -OCIF was digested with restriction enzymes Nde I and Xho I. A DNA fragment with an approximate size of 4.0 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated DNA solution 12. Two microliters of DNA solution 11, 3 μ l of DNA solution 12 and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation was carried out. Competent *E. coli* DH5 α cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR1.

The DNA fragment which is contained in solution G (20 μ l) was digested with restriction enzymes Nde I and Xho I. A DNA fragment with an approximate size of 500 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA

solution was designated as DNA solution 13. Two microliters of DNA solution 13, 3 μ l of DNA solution 12 and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation was carried out. Competent *E. coli* DH5a cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR2.

The DNA fragment which is contained in solution H (20 μ l) was digested with restriction enzymes Nde I and Xho I. A DNA fragment with an approximate size of 500 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as DNA solution 14. Two microliters of DNA solution 14, 3 μ l of DNA solution 12 and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5 α cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR3.

The DNA fragment which is contained in solution I (20 μ l) was digested with restriction enzymes Xho I and Sph I. A DNA fragment with an approximate size of 900 bp was extracted from a preparative agarose gel with QIAEX gel

extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as DNA solution 15. Two micrograms of pSK+ -OCIF was digested with restriction enzymes Xho I and Sph I. A DNA fragment with an approximate size of 3.6 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as DNA solution 16. Two microliters of DNA solution 15, 3 μ l of DNA solution 16 and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent E. coli DH5 α cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR4.

The DNA fragment which is contained in solution J (20 μ l) was digested with restriction enzymes BstP I and Nde I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as DNA solution 17. Two microliters of DNA solution 17, 3 μ l of DNA solution 8 and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent E. coli DH5 α cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DDD1.

The DNA fragment which is contained in solution K (20 μ l) was digested with restriction enzymes Nde I and BstP I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as DNA solution 18. Two microliters of DNA solution 18, 3 μ l of DNA solution 8 and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5 α cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DDD2.

2) Construction of vectors for expressing the OCIF mutants

pSK-OCIF-DCR1, pSK-OCIF-DCR2, pSK-OCIF-DCR3, pSK-OCIF-DCR4, pSK-OCIF-DDD1 and pSK-OCIF-DDD2 were digested with restriction enzymes Bam HI and Xho I. The Bam HI-Xho I DNA fragment containing entire coding sequence for each OCIF mutant was isolated and dissolved in 20 μ l of sterile distilled water. These DNA solutions that contain the Bam HI-Xho I fragment derived from pSK-OCIF-DCR1, pSK-OCIF-DCR2, pSK-OCIF-DCR3, pSK-OCIF-DCR4, pSK-OCIF-DDD1 and pSK-OCIF-DDD2 were designated DCR1 DNA solution, DCR2 DNA solution, DCR3 DNA solution, DCR4 DNA solution, DDD1 DNA solution and DDD2 DNA solution, respectively. One microliter of pCEP 4 DNA solution and 6 μ l of either DCR1 DNA solution, DCR2 DNA solution, DCR3 DNA solution, DCR4 DNA solution, DDD1 DNA solution or DDD2 DNA solution were independently mixed with 7 μ l of

ligation buffer I of DNA ligation kit ver. 2 and ligation reactions were carried out. Competent *E. coli* DH5 α cells (100 μ l) were transformed with 7 μ l of each ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA in which the DNA fragment with deletions is inserted between the recognition sites of Bam HI and Xho I of pCEP 4 by analyzing the DNA structure. The plasmids containing the cDNA encoding OCIF-DCR1, OCIF-DCR2, OCIF-DCR3, OCIF-DCR4, OCIF-DDD1 and OCIF-DDD2 were designated as pCEP4-OCIF-DCR1, pCEP4-OCIF-DCR2, pCEP4-OCIF-DCR3, pCEP4-OCIF-DCR4, pCEP4-OCIF-DDD1 and pCEP4-OCIF-DDD2, respectively.

iii) Preparation of OCIF with C-terminal domain truncation

(1) mutagenesis of OCIF cDNA

A series of OCIF mutants with deletions of from Cys at amino acid residue 379 to Leu 380, from Ser 331 to Leu 380, from Asp 252 to Leu 380, from Asp 177 to Leu 380, from Arg 123 to Leu 380 and from Cys 86 to Leu 380 was prepared. Positions of the amino acid residues are shown in SEQUENCE NO: 4. These mutants were designated as OCIF-CL, OCIF-CC, OCIF-CDD2, OCIF-CDD1, OCIF-CCR4 and OCIF-CCR3, respectively.

Mutagenesis for OCIF-CL was performed by the two-step PCR as described in EXAMPLE 22-(ii). The primer set for the reaction is shown in Table 12. The nucleotide sequences of the primers are shown in SEQUENCE NO:23, 40, 55, and 56. The final PCR products were precipitated with ethanol, dried under vacuum and dissolved in 40 μ l of sterile distilled water. This DNA solution was designated as solution L.

The DNA fragment which is contained in solution L (20 μ l) was digested with restriction enzymes BstP I and EcoR V. A DNA fragment with an approximate size of 100 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as DNA solution 19. Two microliters of DNA solution 19, 3 μ l of DNA solution 10 (described in EXAMPLE 22-(ii)) and 5 μ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent E. coli DH5 α cells were transformed with 5 μ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-CL. Mutagenesis of OCIF cDNA to prepare OCIF-CC, OCIF-CDD2, OCIF-CDD1, OCIF-CCR4 and OCIF-CCR3 was performed by a one-step PCR. PCR reactions for mutagenesis to prepare OCIF-CC, OCIF-CDD2, OCIF-CDD1, OCIF-CCR4 and OCIF-CCR3

10X Ex Taq Buffer (Takara Shuzo)	10 μ l
2.5 mM solution of dNTPs	8 μ l
the plasmid vector containing the entire OCIF cDNA described in EXAMPLE 11 (8ng/ml)	2 μ l
sterile distilled water	73.5 μ l
20 μ M solution of primer OCIF Xho F	5 μ l
100 μ M solution of primer (for mutagenesis)	1 μ l
Ex Taq (Takara Shuzo)	0.5 μ l

Table 12

mutants	primer-1	primer-2	primer-3	primer-4
OCIF-CL	IF 6	CL R	IF 14	CL F

Specific primers were used for each mutagenesis and other components were unchanged.

Primers used for the mutagenesis are shown in Table 13. Their nucleotide sequences are shown in SEQUENCE NO:57-61. The components of each PCR were mixed in a microcentrifuge tube and PCR was performed as follows. The microcentrifuge tubes were treated for 3 minutes at 97 °C and then incubated sequentially, for 30 seconds at 95 °C, 30 seconds at 50 °C and 3 minutes at 70 °C. This three-step incubation procedure was repeated 25 times, and after that, the tubes were incubated for 5 minutes at 70 °C. An aliquot of the reaction mixture was removed from each tube and analyzed by an agarose gel electrophoresis to confirm the size of each product.

The size of the PCR products was confirmed on an agarose gel. Excess primers in the PCRs were removed using Amicon microcon (Amicon) after completion of the reaction. The DNA fragments were precipitated with ethanol, dried under vacuum and dissolved in 40 μ l of sterile distilled water. The DNA fragment in each DNA solution was digested with restriction enzymes Xho I and Bam HI. After the reactions, DNA was precipitated with ethanol, dried under vacuum and dissolved in 20 μ l of sterile distilled water.

The solutions containing DNA fragment with the CC deletion, the CDD2

deletion, the CDD1 deletion, the CCR4 deletion and the CCR3 deletion were designated as CC DNA solution, CDD2 DNA solution, CDD1 DNA solution, CCR4 DNA solution and CC R3 DNA solution, respectively.

Table 13

mutants	primers for the mutagenesis
OCIF-CC	CC R
OCIF-CDD2	CDD2 R
OCIF-CDD1	CDD1 R
OCIF-CCR4	CCR4 R
OCIF-CCR3	CCR3 R

(2) Construction of vectors for expressing the OCIF mutants

pSK-OCIF-CL was digested with restriction enzymes Bam HI and Xho I. The Bam HI-Xho I DNA fragment containing the entire coding sequence for OCIF-CL was isolated and dissolved in 20 μ l of sterile distilled water. This DNA solution was designated as CL DNA solution. One microliter of pCEP 4 DNA solution and 6 μ l of either of CL DNA solution, CC DNA solution, CDD2 DNA solution, CDD1 DNA solution, CCR4 DNA solution or CCR3 DNA solution were independently mixed with 7 μ l of ligation buffer I of DNA ligation kit ver. 2 and ligation reactions were carried out. Competent E. coli DH5 α cells (100 μ l) were transformed with 7 μ l of each ligation mixture. Ampicillin-resistant transformants were screened for clones containing plasmids which have the

desirable mutations in OCIF cDNA by analyzing the DNA structure. In each plasmid, OCIF cDNA fragment having a deletion were inserted between the recognition sites of Xho I and Bam HI of pCEP 4. The plasmids containing the cDNA encoding OCIF-CL, OCIF-CC, OCIF-CDD1, OCIF-CDD2, OCIF-CCR4 and OCIF-CCR3 were designated pCEP4-OCIF-CL, pCEP4-OCIF-CC, pCEP4-OCIF-CDD2, pCEP4-OCIF-CDD1, pCEP4-OCIF-CCR4 and pCEP4-OCIF-CCR3, respectively.

iv) Preparation of OCIF mutants with C-terminal truncation

(1) Introduction of C-terminal truncation to OCIF

A series of OCIF mutants with C-terminal truncation was prepared. OCIF mutant in which 10 residues of from Gln at 371 to Leu at 380 are replaced with 2 residues of Leu-Val was designated OCIF-CBst. OCIF mutant in which 83 residues of from Cys 298 to Leu 380 are replaced with 3 residues of Ser-Leu-Asp was designated OCIF-CSph. OCIF mutant in which 214 residues of from Asn 167 to Leu 380 are removed was designated OCIF-CBsp. OCIF mutant in which 319 residues of from Asp 62 to Leu 380 are replaced with 2 residues of Leu-Val was designated OCIF-CPst. Positions of the amino acid residues are shown in SEQUENCE NO: 4.

Two micrograms each of pSK + -OCIF was digested with one of the restriction enzymes, Bst PI, Sph I, PstI (Takara Shuzo), and Bsp EI (New England Biolabs), and followed by phenol extraction and ethanol precipitation. The precipitated DNA was dissolved in 10 μ l of sterile distilled water. Ends of the DNAs in 2 μ l of each solution were blunted using a DNA blunting kit in final volumes of 5 μ l. To the reaction mixtures, 1 μ g (1 μ l) of an Amber

codon-containing Xba I linker (5'-CTAGTCTAGACTAG-3') and 6 μ l of ligation buffer I of DNA ligation kit ver. 2 were added.

After the ligation reactions, 6 μ l each of the reaction mixtures was used to transform *E. coli* DH5 α . Ampicillin-resistant transformants were screened for clones containing plasmids. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmids thus obtained were named pSK-OCIF-CBst, pSK-OCIF-CSph, pSK-OCIF-CBsp and pSK-OCIF-CPst, respectively.

(2) Construction of vectors for expressing the OCIF mutants

pSK-OCIF-CBst, pSK-OCIF-CSph, pSK-OCIF-CBsp and pSK-OCIF-CPst were digested with restriction enzymes Bam HI and Xho I. The 1.5 kb of DNA fragment containing entire coding sequence for each OCIF mutant was isolated and dissolved in 20 μ l of sterile distilled water. These DNA solutions that contain the Bam HI-XhoI fragment derived from pSK-OCIF-CBst, pSK-OCIF-CSph, pSK-OCIF-CBsp and pSK-OCIF-CPst were designated as CBst DNA solution, CSph DNA solution, CBsp DNA solution and CPst DNA solution, respectively. One microliter of pCEP 4 DNA solution (described in EXAMPLE 22-ii)) and 6 μ l of either CBst DNA solution, CSph DNA solution, CBsp DNA solution or CPst DNA solution were independently mixed with 7 μ l of ligation buffer I of DNA ligation kit ver. 2 and ligation reactions were carried out. Competent *E. coli* DH5 α cells (100 μ l) were transformed with 7 μ l of each ligation mixture. Ampicillin-resistant transformants were screened for clones containing plasmids in which cDNA fragment is inserted between the recognition sites of Bam HI and Xho I of pCEP 4 by analyzing the DNA structure. The plasmids containing the cDNA encoding OCIF-CBst, OCIF-CSph, OCIF-CBsp and OCIF-CPst

were designated as pCEP4-OCIF-CBst, pCEP4-OCIF- CSph, pCEP4-OCIF-CBsp and pCEP4-OCIF-CPst, respectively.

v) Preparation of vectors for expressing the OCIF mutants

E. coli clones harboring the expression vectors for OCIF mutants (total of 21 clones) were grown and the vectors were purified by QIAGEN column (QIAGEN). All the expression vectors were precipitated with ethanol and dissolved in appropriate volumes of sterile distilled water and used for further manipulations shown below.

vi) Transient expression of the cDNAs for OCIF mutants and biological activities of the mutants

OCIF mutants were produced using the expression vectors prepared in EXAMPLE 22-v). The method was essentially the same as described in EXAMPLE 13. Only the modified points are described below. A 24-well plate was used for the DNA transfection. 2×10^5 cells of 293/EBNA suspended in IMDM containing 10% fetal bovine serum were seeded into each well of the plate. One microgram of purified vector DNA and $4 \mu\text{l}$ of lipofectamine were used for each transfection. Mixture of an expression vector and lipofectamine in OPTI-MEM (GIBCO BRL) in a final volume of 0.5 ml was added to the cells in a well. After the cells were incubated at 37°C for 24 hr in a CO_2 incubator, the medium was replaced with 0.5 ml of Ex-cell 301 medium (JSR). The cells were incubated at 37°C for 48 more hours in the CO_2 incubator. The conditioned medium was collected and used for assay for in vitro biological activity. The nucleotide sequences of cDNAs for the OCIF mutants are shown in SEQUENCE NO:83-103. The deduced amino acid sequences for the OCIF mutants are shown in SEQUENCE NO:

62-82. The assay for in vitro biological activity was performed as described in EXAMPLE 13. Antigen concentration of each conditioned medium was determined by ELISA as described in EXAMPLE 24. Table 14 shows specific activity of the mutants relative to that of the unaltered OCIF.

Table 14

mutants	activity
the unaltered OIF	++
OCIF-C19S	+
OCIF-C20S	±
OCIF-C21S	±
OCIF-C22S	+
OCIF-C23S	++
OCIF-DCR1	±
OCIF-DCR2	±
OCIF-DCR3	±
OCIF-DCR4	±
OCIF-DDD1	+
OCIF-DDD2	±
OCIF-CL	++
OCIF-CC	++
OCIF-CDD2	++
OCIF-CDD1	+
OCIF-CCR4	±
OCIF-CCR3	±
OCIF-CBst	++

OCIF-CSph	++
OCIF-CBsp	±
OCIF-CPst	±

++ indicates relative activity more than 50% of that of the unaltered OCIF
+ indicates relative activity between 10% and 50% ± indicates relative activity less than 10%, or production level too low to determine the accurate biological activity

vii) western blot analysis

Ten microliters of the final conditioned medium was used for western blot analysis. Ten microliters of the sample were mixed with 10 µl of SDS-PAGE sample buffer (0.5 M Tris-HCl, 20% glycerol, 4% SDS, 20 µg/ml bromo phenol blue, pH 6.8) boiled for 3 min. and subjected to a 10 % SDS polyacryl amide gel electrophoresis under non-reducing conditions. After the electrophoresis, the separated proteins were blotted to PVDF membrane (ProBlott^R, Perkin Elmer) using a semi-dry electroblotter (BIO-RAD). The membrane was incubated at 37°C with horseradish peroxidase labeled anti-OCIF antibodies for 2 hr. After the membrane was washed, protein bands which react with the labeled antibodies were detected using ECL system (Amersham). Two protein bands with approximate molecular masses of 60kD and 120kD were detected for the unaltered OCIF. On the other hand, almost exclusively 60kD protein band was detected for OCIF-C23S, OCIF-CL and OCIF CC. Protein bands with an approximate masses of 40kD-50kD and 30kD-40kD were the major ones for OCIF-CDD2 and OCIF-CDD1, respectively. These results indicate that Cys at 379 is responsible for the

dimer formation, both the monomers and the dimers maintain the biological activity and a deletion of residues from Asp at 177 to Leu at 380 does not abolish the biological activity of OCIF (positions of the amino acid residues shown in SEQUENCE NO: 4).

EXAMPLE 23

Isolation of human genomic OCIF gene

i) Screening of a human genomic library

An amplified human placenta genomic library in Lambda FIX II vector purchased from STRATAGENE was screened for the gene encoding human OCIF using the human OCIF cDNA as a probe. Essentially, screening was done according to the instruction manual supplied with the genomic library. The basic protocols described in Molecular Cloning: A Laboratory Manual also were employed to manipulate phage, E. coli, and DNA.

The library was titered, and 1×10^6 pfu of phage was mixed with XL1-Blue MRA host E. coli cells and plated on 20 plates (9 cm x 13 cm) with 9 ml per plate of top agarose. The plates were incubated overnight at 37°C. Filter plaque lifts were prepared using Hybond-N nylon membranes (Amersham). The membranes were processed by denaturation in a solution containing 1.5 M NaCl and 0.5 M NaOH for 1 minute at room temperature. The membranes were then neutralized by placing successively for one minute each in 1 M Tris-HCl (pH 7.5) and a solution containing 1.5 M NaCl and 0.5 M Tris-HCl (pH 7.5). The membranes were then transferred onto a filter paper wet with 2xSSC. Phage DNA was fixed on the membranes with 1200 μ Joules of UV energy in STRATALINKER UV crosslinker 2400 (STRATAGENE) and the membranes were air dried. The membranes were immersed in Rapid Hybridization buffer (Amersham) and incubated for one hour at 65 °C before hybridization with 32 P-labeled cDNA probe in the same buffer overnight at 65°C. Screening probe was prepared by labeling the

OCIF cDNA with ^{32}P using the Megaprime DNA labeling system (Amersham). Approximately, 5×10^5 cpm probe was used for each ml of hybridization buffer. After the hybridization, the membranes were rinsed in 2xSSC for five minutes at room temperature. The membranes were then washed four times, 20 minutes each time, in 0.5xSSC containing 0.1 % SDS at 65 °C. After the final wash, the membranes were dried and subjected to autoradiography at -80 °C with SUPER HR-H X-ray film (FUJI PFOTO FILM Co., Ltd.) and an intensifying screen. Upon examination of the autoradiograms, six positive signals were detected. Agar plugs were picked from the regions corresponded to these signals for phage purification. Each agar plug was soaked overnight in 0.5 ml of SM buffer containing 1% chloroform to extract phage. Each extract containing phage was diluted 1000 fold with SM buffer and an aliquot of 1 ml or 20 ml was mixed with host E. coli described above. The mixture was plated on agar plates with top agarose as described above. The plates were incubated overnight at 37 °C, and filter lifts were prepared, prehybridized, hybridized, washed and autoradiographed as described above. This process of phage purification was applied to all six positive signals initially detected on the autoradiograms and was repeated until all phage plaques on agar plates hybridize with the cDNA probe. After purification, agar plugs of each phage isolate were soaked in SM buffer containing 1% chloroform and stored at 4 °C. Six individual phage isolates were designated λ OIF3, λ OIF8, λ OIF9, λ OIF11, λ OIF12 and λ OIF17, respectively.

ii) Analysis of the genomic clones by restriction enzyme digestion and

Southern blot hybridization

DNA was prepared from each phage isolate by the plate lysate method as described in Molecular Cloning: A Laboratory Manual. DNA prepared from each phage was digested with restriction enzymes and the fragments derived from the digestion were separated on agarose gels. The fragments were then transferred to nylon membranes and subjected to Southern blot hybridization using OCIF cDNA as a probe. The results of the analysis revealed that the six phage isolates are individual clones. Among these fragments derived from the restriction enzyme digestion, those fragments which hybridized with the OCIF cDNA probe were subcloned into plasmid vectors and subjected to the nucleotide sequence analysis as described below.

iii) Subcloning restriction fragments derived from genomic clones into plasmid vectors and determination of the nucleotide sequence.

λ OIF8 DNA was digested with restriction enzymes EcoRI and NotI, and the DNA fragments derived from these were separated on a 0.7% agarose gel. The 5.8 kilobase pairs (kb) EcoRI/NotI fragment was extracted from the gel using QIAEX II Gel Extraction Kit (QIAGEN) according to the procedure recommended by the manufacturer. The 5.8 kb EcoRI/NotI fragment was ligated with pBluescript II SK+ vector (STRATAGENE) which had been linearized with restriction enzymes EcoRI and NotI, using Ready-To-Go T4 DNA Ligase (Pharmacia) according to the procedure recommended by the manufacturer. Competent DH5 α E. coli cells (Amersham) were transformed with the recombinant plasmid and transformants were selected on L-plates containing 50 μ g/ml of ampicillin.

A clone harboring the recombinant plasmid containing the 5.8 kb EcoRI/NotI fragment was isolated and this plasmid was termed pBSG8-5.8. pBSG8-5.8 was digested with HindIII and 0.9 kb of DNA fragment derived from this digestion was isolated in the same manner as described above. This 0.9 kb fragment was then cloned in pBluescript II SK- at the HindIII site as described above. This recombinant plasmid containing 0.9 kb HindIII fragment was denoted pBS8H0.9.

λ OIF11 DNA was digested with EcoRI and 6 kb, 3.6 kb, 2.6 kb EcoRI fragments were isolated in the same manner as described above and cloned in pBluescript II SK+ vector at the EcoRI site as described above. These recombinant plasmids were termed pBSG11-6, pBSG11-3.6, and pBSG11-2.6, respectively. pBSG11-6 was digested with HindIII and the digest was applied on a 0.7 % agarose gel. Three fragments, 2.2 kb, 1.1 kb, and 1.05 kb in length, were extracted from the gel and cloned independently in pBluescript II SK- vector at the HindIII site in the same manner as described above. These recombinant plasmids were termed pBS6H2.2, pBS6 H1.1 and pBS6H1.05, respectively.

The nucleotide sequence of the cloned genomic DNA was determined using ABI Dyedexoxy Terminator Cycle Sequencing Ready Reaction Kit (PERKIN ELMER) and 373A DNA Sequencing system (Applied Biosystems). Plasmids pBSG8-5.8, pBS8H0.9, pBSG11-6, pBSG11-3.6, pBSG11-2.6, pBS6H2.2, pBS6H1.1 and pBS6H1.05 were prepared according to the alkaline-SDS procedure as described in Molecular Cloning: A Laboratory Manual and used as templates for the DNA sequence analysis. Nucleotide sequence of the human OCIF gene was presented in Sequence No 104 and Sequence No 105. The nucleotide sequence of the DNA,

between exon 1 and exon 2 was not entirely determined. There is a stretch of approximately 17 kb of nucleotides between the sequences given in sequence No. 104 and sequence No. 105.

EXAMPLE 24

Quantitation of OCIF by EIA

i) Preparation of anti-OCIF antibody

Male JW rabbits (Kitayama LABES Co., LTD) weighing 2.5-3.0 kg were used for immunization for preparing antisera. Three male JW rabbits (Kitayama LABES Co., LTD) weighing 2.5-3.0 kg were used for immunization. For immunization, emulsion was prepared by mixing an equal volume of rOCIF (200 μ g/ml) and complete Freund's adjuvant (Difco, Cat. 0638-60-7). The rabbits were immunized subcutaneously six times at the interval of one week with 1 ml of emulsion per injection. The rabbits were injected six times at the interval of seven days subcutaneously. Whole blood was obtained ten days after the final immunization and serum was separated. Antibody was purified from serum as follows. Antiserum was diluted two-fold with PBS. After adding ammonium sulfate at a final concentration of 40 w/v %, antiserum was allowed to stand at 4 °C for 1 hr.. Precipitate obtained by centrifugation at 8000 x g for 20 min. was dissolved in a small volume of PBS and was dialyzed against PBS. The resulting solution was loaded onto a Protein G-Sepharose column (Pharmacia). After washing with PBS, absorbed immunoglobulin G was eluted with 0.1 M glycine-HCL buffer (pH 3.0). Elutes were neutralized with 1.5 M Tris-HCL buffer (pH 8.7) immediately and were dialyzed against PBS. Protein

concentration was determined by absorbance at 280nm ($E_{1\%}^{1\text{cm}}$ 13.5).

Horseradish peroxidase labeled antibody was prepared using ImmunoPure Maleimide Activated Horseradish Peroxidase Kit (Pierce, Cat.31494). Briefly, one mg of IgG was incubated with 80 ug of N-succinimidyl-S-acetylthioacetate for 30 min. After deacetylation with 5 mg of hydroxylamine HCl, modified IgG was separated by polyacrylamide desalting column. Protein pool mixed with one mg of maleimide activated horseradish peroxidase was incubated at room temperature for 1 hr.

ii) Quantitation of OCIF by sandwich EIA

Microtiter plates (Nunc MaxiSorp Immunoplate) were coated with rabbit anti-OCIF IgG by incubating 0.2 ug in 100 ul of 50 mM sodium bicarbonate buffer pH 9.6 at 4C overnight. After blocking the plates by incubating for 1 hour at 37°C with 300 ul of 25% BlockAce/PBS (Snow Brand Milk Products), 100ul of samples were incubated for 2 hours at room temperature. After washing the plates three times with PBST (PBS containing 0.05% Tween20), 100 ul of 1:10000 diluted horseradish peroxidase labeled anti-OCIF IgG was added and incubated for 2 hours at room temperature. The amount of OCIF was determined by incubation with 100 ul of a substrate solution (TMB, ScyTek Lab., Cat.TM4999) and measurement of the absorbance at 450 nm using an ImmunoReader (Nunc NJ2000). Purified recombinant OCIF was used as a standard protein and a typical standard curve was shown in Fig. 13.

EXAMPLE 25

Anti-OCIF monoclonal antibody

i) Preparation of hybridoma producing anti-OCIF monoclonal antibody.

OCIF was purified to homogeneity from culture medium of human fibroblasts, IMR-90 by the purification method described in Example 11. Purified OCIF was dissolved in PBS at a concentration of $10 \mu\text{g}/100 \mu\text{l}$. BALB/c mice were immunized by administering this solution intraperitoneally three times every two weeks. In the first and the second immunizations, the emulsion composed of an equal volume of OCIF and Freund's complete adjuvant was administered. Three days after the final administration, the spleen was taken out, lymphocytes were isolated and fused with mouse myeloma p3x63-Ag8.653 cells according to the conventional method using polyethyleneglycol. Then the fused cells were cultured in HAT medium to select hybridoma. Subsequently, to check whether the selected hybridomas produce anti-OCIF antibody, anti-OCIF antibody in each culture medium of hybridomas was determined by solid phase ELISA which was prepared by coating each well in 96-well immunoplates (Nunc) with $100 \mu\text{l}$ of purified OCIF ($10 \mu\text{g}/\text{ml}$ in 0.1 M NaHCO_3) and by blocking each well with 50% BlockAce (Snow Brand Milk Products Co. Ltd.). The hybridoma clones secreting anti-OCIF antibody were established by cloning 3 - 5 times by limit dilution and by screening using the above solid phase ELISA. Among thus obtained hybridoma clones, several hybridoma clones with high production of anti-OCIF antibody were selected.

ii) Production of anti-OCIF monoclonal antibodies.

Each hybridoma clone secreting anti-OCIF antibody, which was obtained in

EXAMPLE 25-i), was transplanted intraperitoneally to mice given Pristane (Aldrich) at a cell density of 1×10^6 cells/mouse. The accumulated ascites was collected 10 - 14 days after the transplantation and the ascites containing anti-OCIF specific monoclonal antibody of the present invention was obtained. Purified antibodies were obtained by Affigel protein A Sepharose

chromatography (BioRad) according to the manufacturer's manual. That is, the ascites was diluted with equal volume of a binding buffer (BioRad) and applied to protein A column. The column was washed with a sufficient volume of the binding buffer and eluted with an elution buffer (BioRad). After neutralizing, the obtained eluate was dialyzed in water and subsequently lyophilized. The purity of the obtained antibody was analyzed by SDS/PAGE and a homogenous band with a molecular weight of about 150,000 was detected.

iii) Selection of monoclonal antibody having high affinity to OCIF

Each antibody obtained in EXAMPLE 25-ii) was dissolved in PBS and the concentration of protein in the solution was determined by the method of Lowry. Each antibody solution with the same concentration was prepared and then serially diluted with PBS. Monoclonal antibodies, which can recognize OCIF even at highly diluted solution, were selected by solid phase ELISA described in EXAMPLE 25-ii). Thus three monoclonal antibodies A1G5, E3H8 and D2F4 can be selected.

iv) Determination of class and subclass of antibodies

The class and subclass of the antibodies of the present invention obtained in EXAMPLE 25-iii) were analyzed using an immunoglobulin class and subclass analysis kit (Amersham). The procedure was carried out according to the protocol disclosed in the directions. The results were shown in Table 15. The antibodies of the present invention, E3H8, A1G5 and D2F4 belong to IgG₁, IgG_{2a} and IgG_{2b}, respectively.

Table 15

Analysis of class and subclass of the antibodies in the present invention.

Antibody	IgG ₁	IgG _{2a}	IgG _{2b}	IgG ₃	IgA	IgM	κ
A1G5	—	+	—	—	—	—	+
E3H8	+	—	—	—	—	—	+
D2F4	—	—	+	—	—	—	+

v) Determination of OCIF by ELISA

Three kinds of monoclonal antibodies, A1G5, E3H8 and D2F4, which were obtained in EXAMPLE 25-iv), were used as solid phase antibodies and enzyme-labeled antibodies, respectively. Sandwich ELISA was constructed by each combination of solid phase antibody and labeled antibody. The labeled antibody was prepared using Immuno Pure Maleimide Activated Horseradish Peroxidase Kit (Pierce, Cat. No. 31494). Each monoclonal antibody was

dissolved in 0.1 M NaHCO_3 at a concentration of 10 $\mu\text{g/ml}$, and 100 μl of the solution was added to each well in 96-well immunoplates (Nunc, MaxiSorp Cat. No. 442404) followed by allowing to stand at room temperature overnight. Subsequently, each well in the plates was blocked with 50% Blockace (Snow Brand Milk Products, Co., Ltd.) at room temperature for 50 minutes, and then was washed three times with PBS containing 0.1% Tween 20 (washing buffer).

A series of concentrations of OCIF was prepared by diluting OCIF with 1st reaction buffer (0.2 M Tris-HCl buffer, pH 7.4, containing 40% Blockace and 0.1% Tween 20). Each well in 96-well immunoplates was filled with 100 μl of the prepared OCIF solution with each concentration, allowed to stand at 37 °C for 3 hours, and subsequently washed three times with the washing buffer. For dilution of POD-labeled antibody, 2nd reaction buffer (0.1 M Tris-HCl buffer, pH 7.4, containing 25% Blockace and 0.1% Tween 20) was used. POD-labeled antibody was diluted 400-fold with 2nd reaction buffer, and 100 μl of the diluted solution was added to each well in the immunoplates. Each immunoplate was allowed to stand at 37 °C for 2 hours, and subsequently washed three times with the washing buffer. After washing, 100 μl of a substrate solution (0.1 M citrate-phosphate buffer, pH 4.5, containing 0.4 mg/ml of o-phenylenediamine HCl and 0.006% H_2O_2) was added to each well in the immunoplates and the immunoplates were incubated at 37°C for 15 min. The enzyme reaction was terminated by adding 50 μl of 6 N H_2SO_4 to each well. The optical density of each well was determined at 492 nm using an immunoreader (ImmunoReader NJ 2000, Nunc).

Using three kinds of monoclonal antibody in the present invention, each

combination of solid phase and POD-labeled antibodies leads to a accurate determination of OCIF. Each monoclonal antibody in the present invention was confirmed to recognize a different epitope of OCIF. A typical standard curve of OCIF using a combination of solid phase antibody, A1G5 and POD-labeled antibody, E3H8 was shown in Fig. 14.

vi) Determination of OCIF in human serum

Concentration of OCIF in five samples of normal human serum was determined using an EIA system described in EXAMPLE 25-v). The immunoplates were coated with A1G5 as described in EXAMPLE 25-v), and 50 μ l of 1st. reaction buffer was added to each well in the immunoplates. Subsequently, 50 μ l of each human serum was added to each well in the immunoplates. The immunoplates were incubated at 37°C for 3 hours and then washed three times with the washing buffer. After washing, each well in the immunoplates was filled with 100 μ l of POD-E3H8 antibody diluted 400-fold with 2nd. reaction buffer and incubated at 37°C for 2 hours. After washing the immunoplates three times with the washing buffer, 100 μ l of the substrate solution described in EXAMPLE 25-v) was added to each well and incubated at 37°C for 15 min. The enzyme reaction was terminated by adding 50 μ l of 6 N H₂SO₄ to each well in the immunoplates. The optical density of each well was determined at 492 nm using an immunoreader (ImmunoReader NJ 2000, Nunc).

1st. reaction buffer containing the known amount of OCIF was treated in the same way and a standard curve of OCIF as shown in fig. 2 was obtained. Using the standard curve of OCIF, the amount of OCIF in human serum sample was

determined. The results were shown in Table 14.

Table 14

The amount of OCIF in normal human serum

Serum Sample	OCIF Concentration (ng/ml)
1	5. 0
2	2. 0
3	1. 0
4	3. 0
5	1. 5

EXAMPLE 26

Therapeutic effect on osteoporosis

(1) Method

Male Fischer rats, 6 weeks-old, were subjected to denervation of left forelimb. These rats were assigned to four groups(10 rats/group) and treated as follows ; group A, sham operated rats without administration ; group B, denervated rats with intravenous administration of vehicle ; group C, denervated rats administered OCIF intravenously at a dose of 5 μ g/kg twice a day ; group D, denervated rats administered OCIF intravenously at a dose of 50 μ g/kg twice a day. After denervation, OCIF was administered daily for 14 days. After 2 weeks treatment, the animals were sacrificed and their forelimbs were dissected. Thereafter bones were tested for mechanical

strength.

(2) Results

Decrease of bone strength was observed in the animals of control groups as compared to those animals of the normal groups while bone strength was increase in the groups of animal received 50 mg of OCIF per kg body weight.

Industrial availability

The present invention provides both a novel protein which inhibits formation of osteoclasts and a efficient procedure to produce the protein. The protein of the present invention has an activity to inhibit formation of osteoclasts. The protein will be useful for the treatment of many diseases accompanying bone loss, such as osteoporosis, and as an antigen to be used for the immunological diagnosis of such diseases.

Referring to the deposited the microorgainsm

Name and Address of the Depositary Authority

Name: National Institute of Bioscience and Human-Technology
Agency of Industrial Science and Technology
Ministry of International Trade and Industry

Address: 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken
305, JAPAN

Deposited date: June 21, 1995

(It was transferred from Bikkoken No. P-14998, which was deposited on June 21, 1995. Transferred date: October 25, 1995)

Acession Number: FERM BP-5267

Claims

1. A protein characterized by the following properties:

(a) molecular weights on SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

; approximately 60 kD under reducing conditions

; approximately 60 kD and 120 kD under non-reducing conditions

(b) a high affinity to cation-exchange column and heparin column

(c) a biological activity to inhibit osteoclast differentiation and/or maturation

; its activity is decreased by heating at 70°C for 10 min or at 56°C for 30 min.

; its activity is lost by heating at 90 °C for 10 min

(d) internal amino acid sequences provided in sequence numbers 1, 2, and 3.

2. A protein of claim 1 having N-terminal amino acid sequences provided in sequence number 7.

3. A protein of claim 1 produced in human fibroblasts.

4. A method of producing the protein of claim 1, 2, and 3 by the following process: cultivating human fibroblasts ; purifying the protein by a combination of ion-exchange column, affinity-column and reverse phase-column chromatography.

5. A method of producing the protein of claim 4 by cultivating human fibroblasts on alumina ceramic pieces.

6. A protein with amino acid sequence provided in sequence number 4.

7. cDNAs encoding amino acid sequence provided in sequence number 4.

8. cDNA with nucleotide sequence provided in sequence number 6.
9. cDNAs that hybridize to cDNA provided in sequence number 6 under moderately stringent conditions.
10. A protein expressed from cDNA encoding amino acid sequence provided in sequence number 4.
11. A protein with a biological activity to inhibit osteoclast differentiation and/or maturation, that obtain as amino acid expressed cDNA sharing at least 80 % sequence identity with the amino acid sequence provided in sequence number 4.
12. A method of production of the protein with the following properties and inhibit osteoclast differentiation and/or maturation by gene engineering using cDNA encoding amino acid sequence provided in sequence number 4:
 - (a) molecular weights on SDS-polyacrylamide gel electrophoresis (SDS-PAGE)
 - ; approximately 60 kD under reducing conditions
 - ; approximately 60 kD and 120 kD under non-reducing conditions
 - (b) a high affinity to cation-exchange column and heparin column
 - (c) ; inhibit osteoclast differentiation and/or maturation activity is decreased by heating at 70°C for 10 min or at 56°C for 30 min
 - ; its activity is lost by heating at 90 °C for 10 min
 - (d) internal amino acid sequence provided in sequence number 1-3.
13. A method of producing the protein according to claim 10 by gene engineering using mammalian cells as host cells.
14. A method of producing the protein according to claim 13 by gene engineering using 293/EBNA cells or CHO cells as mammalian host cells.

15. A cDNA with nucleotide sequence provided in sequence number 8.
16. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 8.
17. cDNAs encoding amino acid sequence provided in sequence number 9.
18. A cDNA with nucleotide sequence provided in sequence number 10.
19. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 10.
20. cDNAs encoding amino acid sequence provided in sequence number 11.
21. A cDNA with nucleotide sequence provided in sequence number 12.
22. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 12.
23. cDNAs encoding amino acid sequence provided in sequence number 13.
24. A cDNA with nucleotide sequence provided in sequence number 14.
25. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 14.
26. cDNAs encoding amino acid sequence provided in sequence number 15.
27. A cDNA with nucleotide sequence provided in sequence number 83.
28. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 83.
29. cDNAs encoding amino acid sequence provided in sequence number 62.
30. A cDNA with nucleotide sequence provided in sequence number 84.
31. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 84.
32. cDNAs encoding amino acid sequence provided in sequence number 63.

33. A cDNA with nucleotide sequence provided in sequence number 85.
34. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 85.
35. cDNAs encoding amino acid sequence provided in sequence number 64.
36. A cDNA with nucleotide sequence provided in sequence number 86.
37. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 86.
38. cDNAs encoding amino acid sequence provided in sequence number 65.
39. A cDNA with nucleotide sequence provided in sequence number 87.
40. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 87.
41. cDNAs encoding amino acid sequence provided in sequence number 66.
42. A cDNA with nucleotide sequence provided in sequence number 88.
43. A protein encoded by a cDNA having a sequence provided in sequence number 88.
44. cDNAs encoding amino acid sequence provided in sequence number 67.
45. A cDNA with nucleotide sequence provided in sequence number 89.
46. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 89.
47. cDNAs encoding amino acid sequence provided in sequence number 68.
48. A cDNA with nucleotide sequence provided in sequence number 90.
49. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 90.
50. cDNAs encoding amino acid sequence provided in sequence number 69.

51. A cDNA with nucleotide sequence provided in sequence number 91.
52. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 91.
53. cDNAs encoding amino acid sequence provided in sequence number 70.
54. A cDNA with nucleotide sequence provided in sequence number 92.
55. A protein encoded by a cDNA having a nucleotide sequence provided in number 92.
56. cDNAs encoding amino acid sequence provided in sequence number 71.
57. A cDNA with nucleotide sequence provided in sequence number 93.
58. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 93.
59. cDNAs encoding amino acid sequence provided in sequence number 72.
60. A cDNA with nucleotide sequence provided in sequence number 94.
61. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 94.
62. cDNAs encoding amino acid sequence provided in sequence number 73.
63. A cDNA with nucleotide sequence provided in sequence number 95.
64. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 95.
65. cDNAs encoding amino acid sequence provided in sequence number 74.
66. A cDNA with nucleotide sequence provided in sequence number 96.
67. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 96.
68. cDNAs encoding amino acid sequence provided in sequence number 75.

69. A cDNA with nucleotide sequence provided in sequence number 97.
70. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 97.
71. cDNAs encoding amino acid sequence provided in sequence number 76.
72. A cDNA with nucleotide sequence provided in sequence number 98.
73. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 98.
74. cDNAs encoding amino acid sequence provided in sequence number 77.
75. A cDNA with nucleotide sequence provided in sequence number 99.
76. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 99.
77. cDNAs encoding amino acid sequence provided in sequence number 78.
78. A cDNA with nucleotide sequence provided in sequence number 100.
79. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 100.
80. cDNAs encoding amino acid sequence provided in sequence number 79.
81. A cDNA with nucleotide sequence provided in sequence number 101.
82. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 101.
83. cDNAs encoding amino acid sequence provided in sequence number 80.
84. A cDNA with nucleotide sequence provided in sequence number 102.
85. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 102.
86. cDNAs encoding amino acid sequence provided in sequence number 81.

87. A cDNA with nucleotide sequence provided in sequence number 103.
88. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 103.
89. cDNAs encoding amino acid sequence provided in sequence number 82.
90. Genomic DNAs encoding the amino acid sequence provided in sequence number 4.
91. Genomic DNAs of Claim 90 with the nucleotide sequence provided in sequence number 104 or 105.
92. An antibody having specific affinity to the OCIF
93. An antibody of Claim 92 that is polyclonal antibody.
94. An antibody of Claim 92 that is monoclonal antibody.
95. A monoclonal antibody of Claim 94 being characterized by the following properties.

Molecular weight of about 150,000, and of subclass IgG₁, IgG_{2a}, or IgG_{2b}.
96. A method of determining the concentration of the protein of the OCIF using the antibodies of Claim 92, 93, 94, and 95.

Abstract

A protein which inhibits osteoclast differentiation and/or maturation and a method of production of the protein. The protein is produced by human embryonic lung fibroblasts and has molecular weight of about 60 kD and about 120 kD under non-reducing conditions and about 60 kD under reducing conditions on SDS-polyacrylamide gel electrophoresis, respectively.

The protein can be isolated and purified from culture medium of the said fibroblasts. Furthermore, the protein can be produced by gene engineering.

The present invention includes cDNA for producing the protein by gene engineering, antibodies having specific affinity to the protein or a method for determination of the protein concentration using the antibodies.

Fig. 1

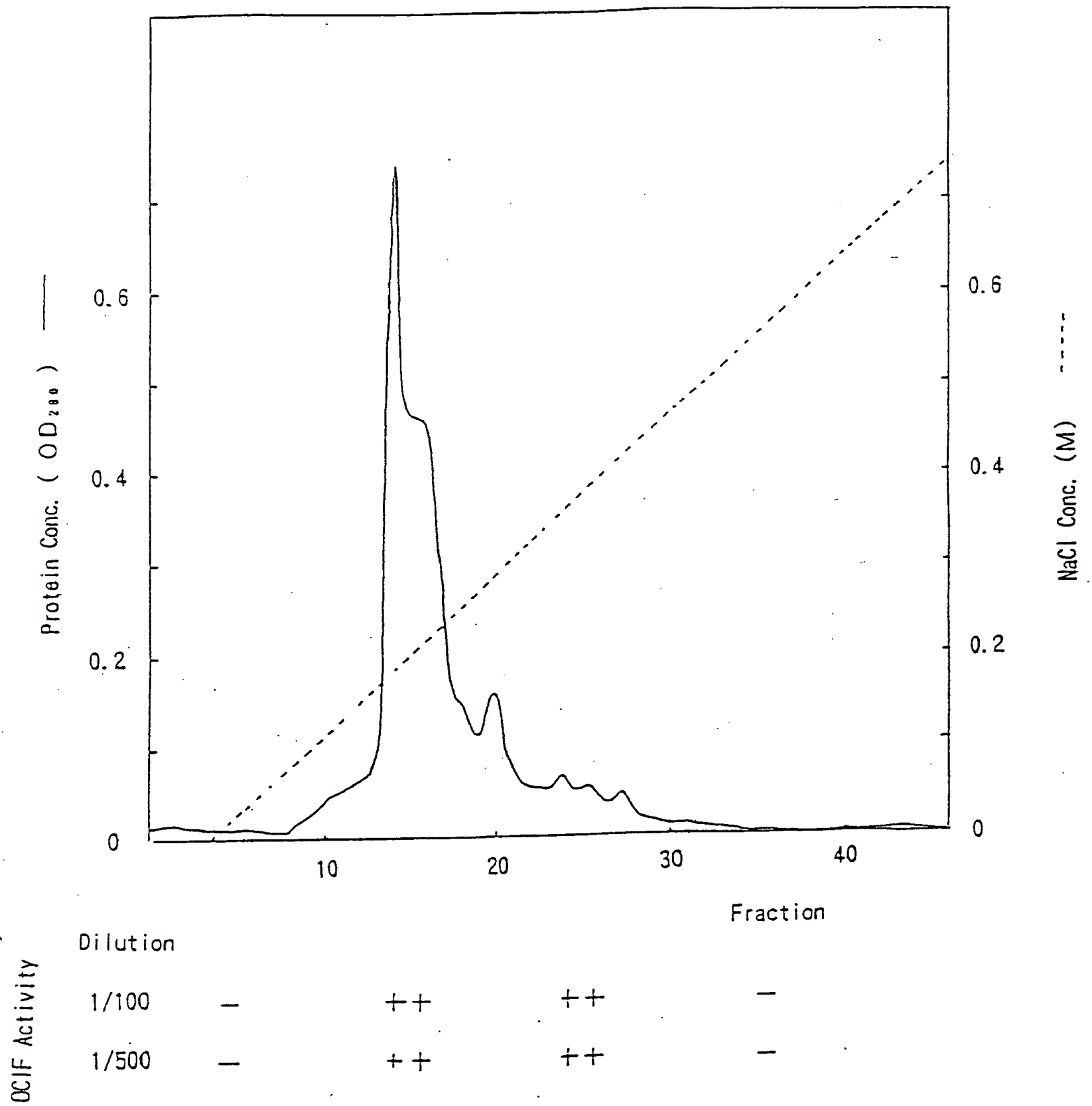


Fig. 2

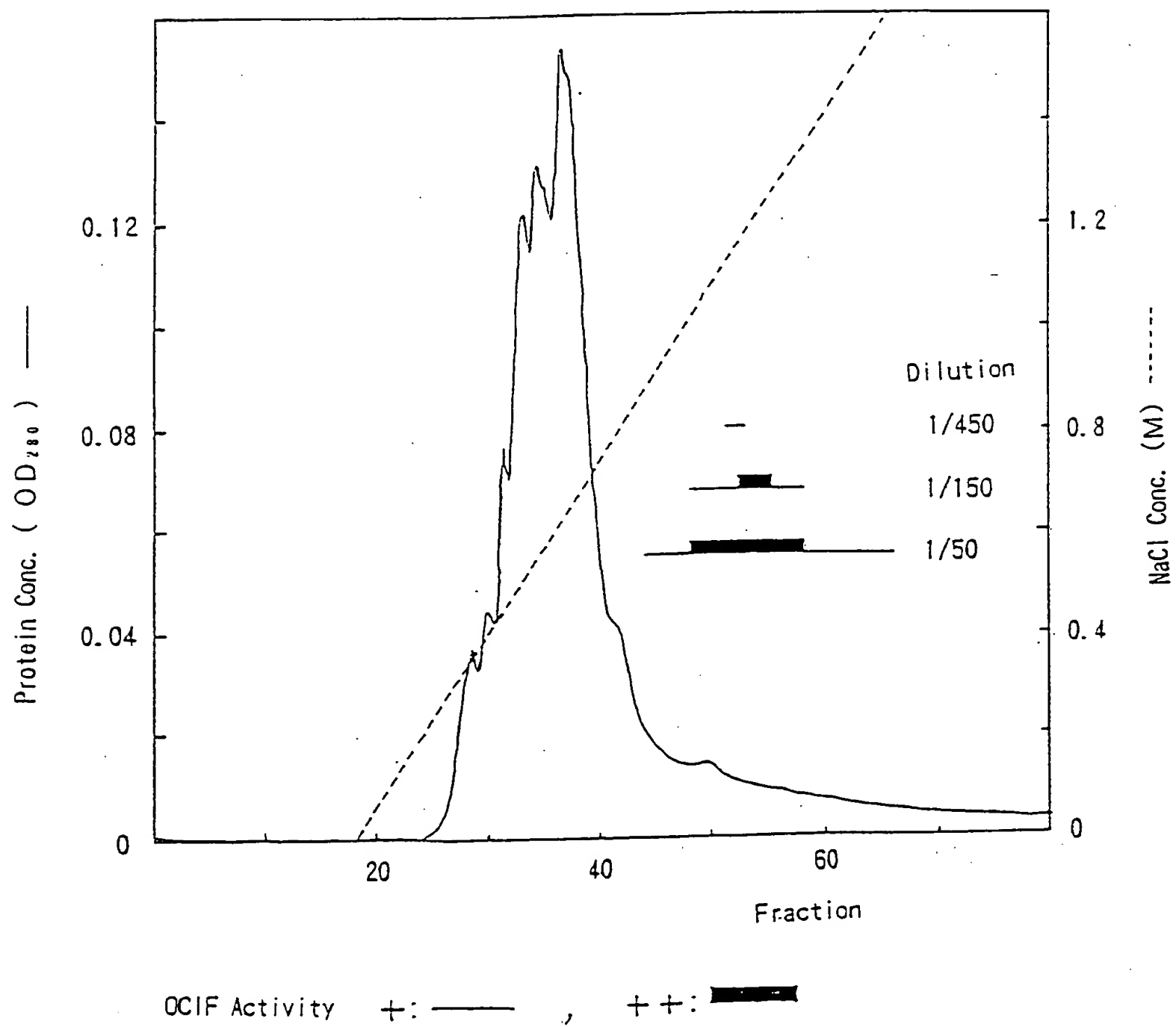


Fig. 3

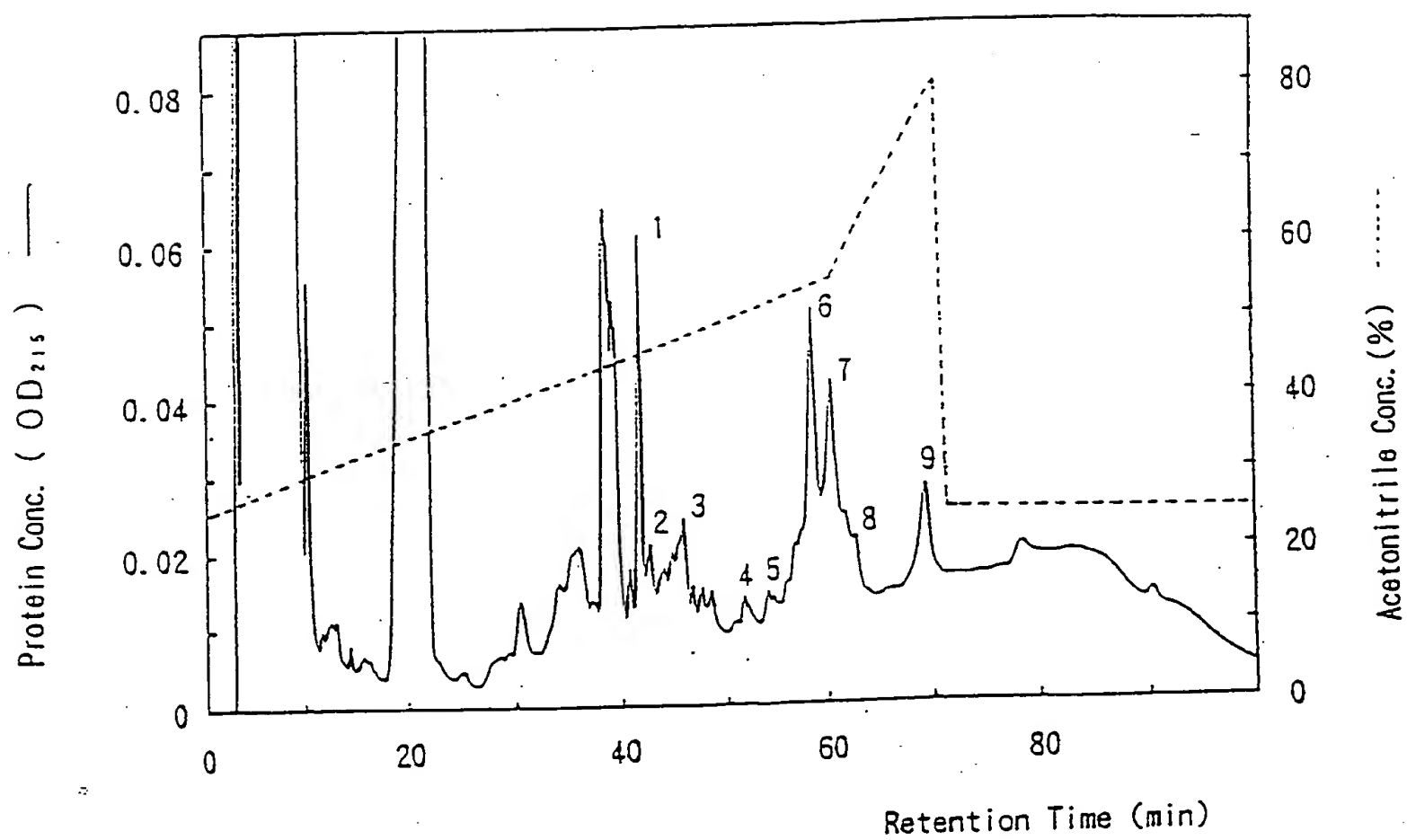


Fig. 4

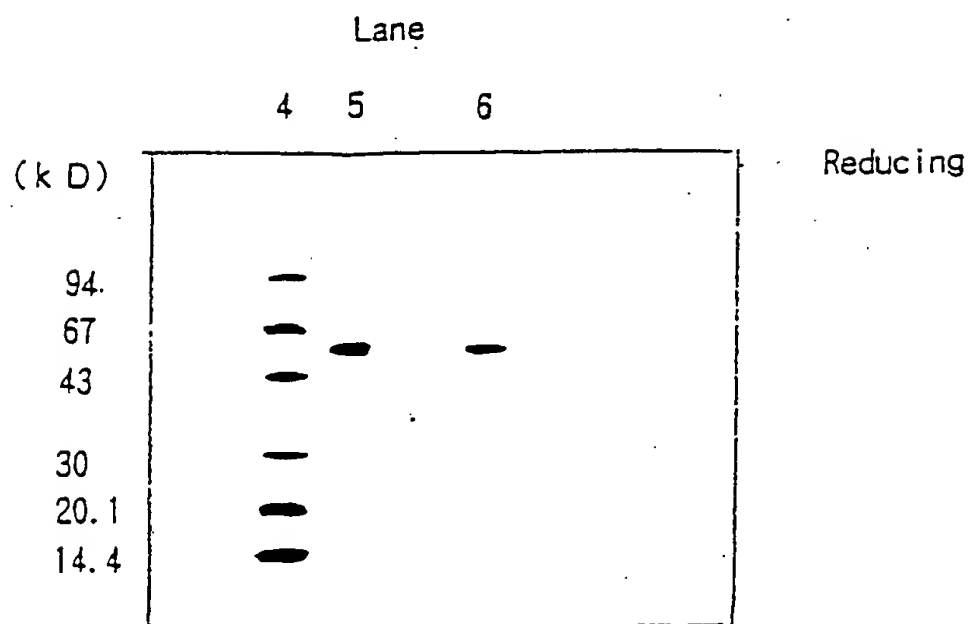
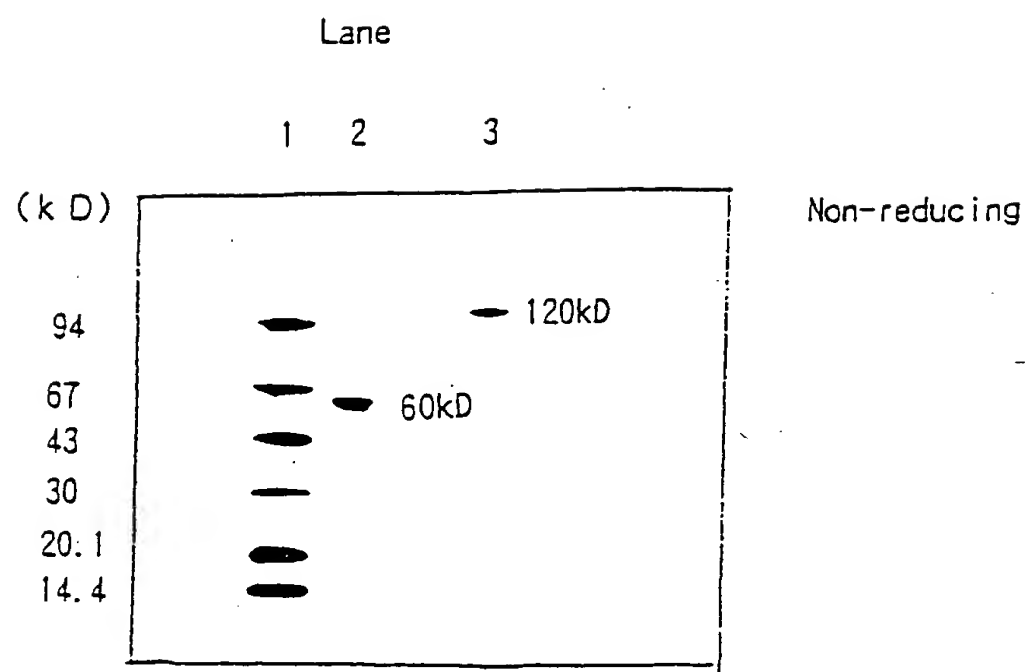


Fig. 5

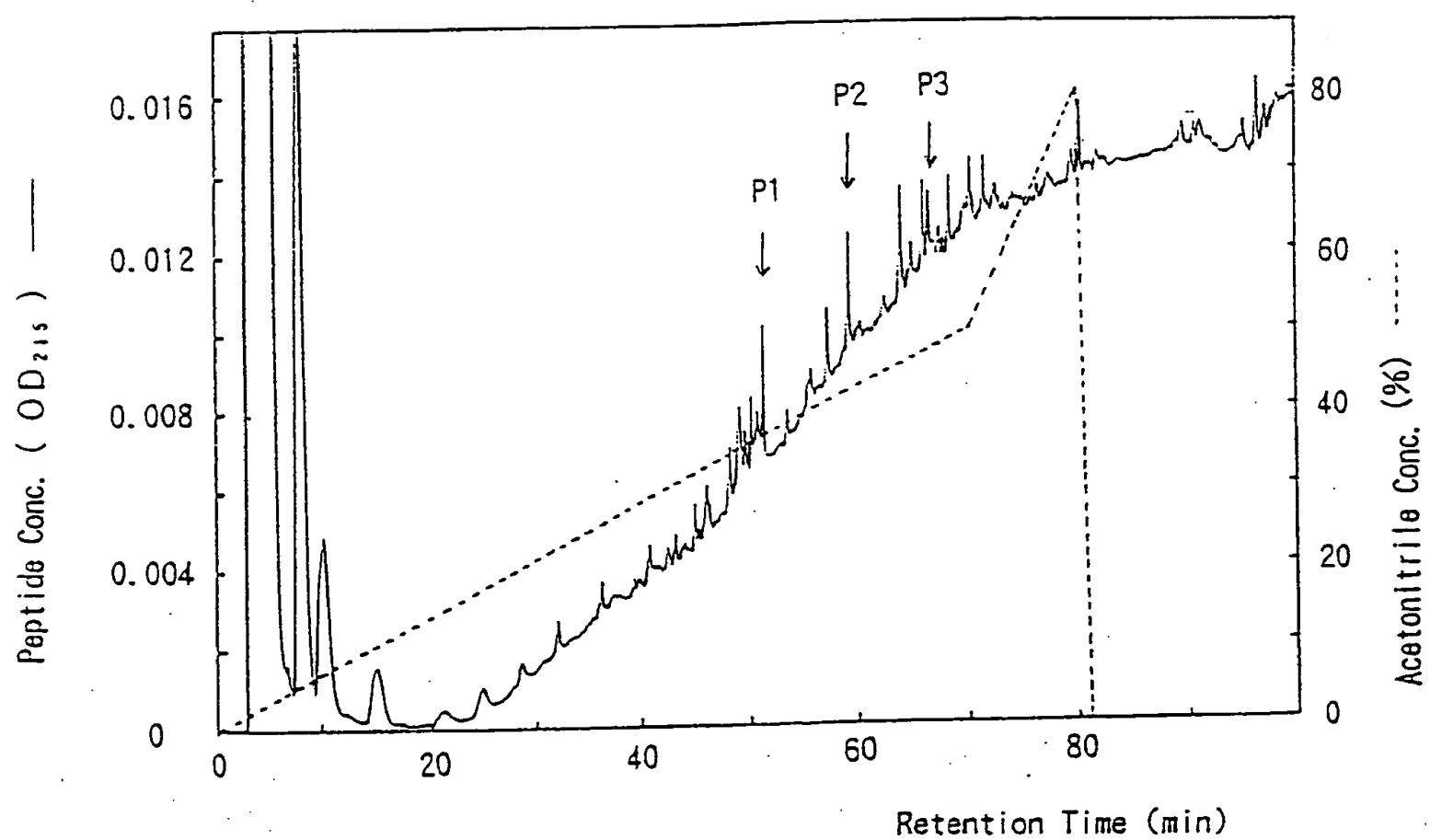


Fig. 6

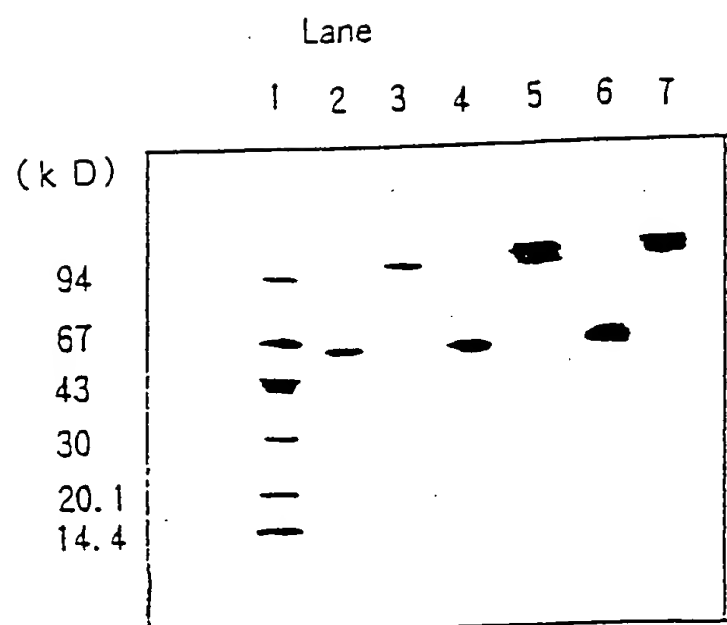


Fig. 7

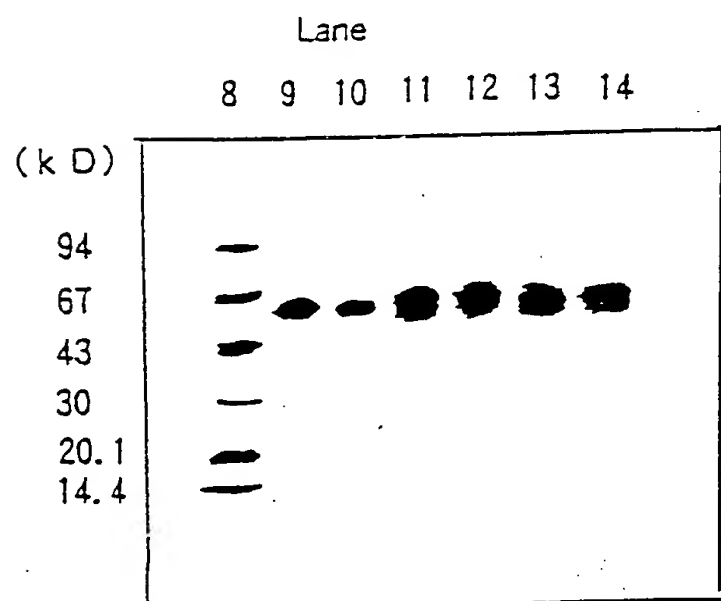


Fig. 8

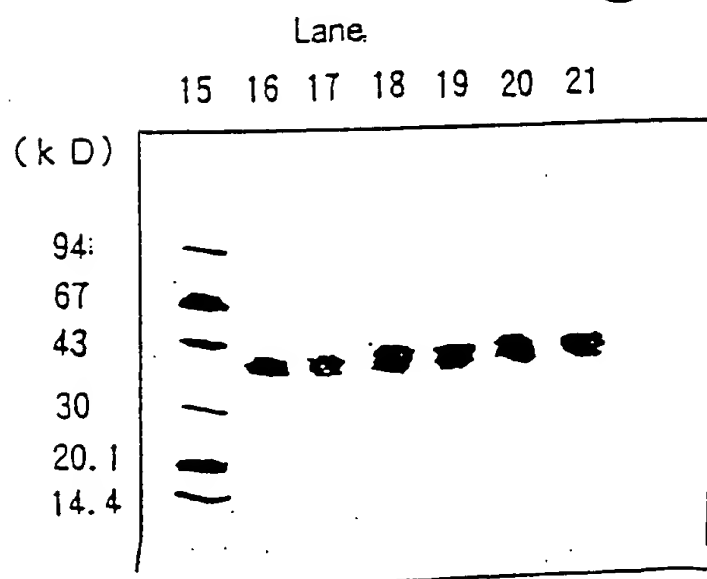


Fig. 9

1
MNNLLCCALVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF1)

MNNLLCCALVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF2)
1

61
VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)

VCAPCPDHYYTDSWHTSDECLYCSPVCKE-----CNRTHNRVCECKEGRYLEIEFCLK (OCIF2)
61

121
HRSCPPGFGVVQAGTPERN TVCKRCPDGGFFSNETSSKAPCRKHTNCSVFGLLLTQKGNAT (OCIF1)

HRSCPPGFGVVQAGTPERN TVCKRCPDGGFFSNETSSKAPCRKHTNCSVFGLLLTQKGNAT (OCIF2)
114

181
HDNICS GNSESTQKCGIDVTLCEEAFRFAVPTKFTPNWLSVLVDNLPGTKVNAESVERI (OCIF1)

HDNICS GNSESTQKCGIDVTLCEEAFRFAVPTKFTPNWLSVLVDNLPGTKVNAESVERI (OCIF2)
174

241
KRQHSSQEQT FQLLKLWKHQNKDQDIVKKIIQDIDLCENSVQRHIGHANLTFEQLRSLME (OCIF1)

KRQHSSQEQT FQLLKLWKHQNKDQDIVKKIIQDIDLCENSVQRHIGHANLTFEQLRSLME (OCIF2)
234

301
SLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALKH SKTYHFPKT (OCIF1)

SLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALKH SKTYHFPKT (OCIF2)
294

361
VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF1)

VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF2)
354

Fig. 10

1
MNNLLCCALVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF1)
** *****
MNKLLCCALVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF3)
1

61
VCAPCPDHYYTDSWHTSDECLYCSVPCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)

VCAPCPDHYYTDSWHTSDECLYCSVPCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF3)
61

121
HRSCPPGFGVVQAGTPERN TVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTQKGNAT (OCIF1)

HRSCPPGFGVVQAGTPERN TVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTQKGNAT (OCIF3)
121

181
HDNICS GNSESTQKCGIDVTLC EEAFFRFAVPTKFTPNWLSVLVDNLPGTKVNAESVERI (OCIF1)

HDNICS GNSESTQKCGIDVTLC EEAFFRFAVPTKFTPNWLSVLVDNLPGTKVNAESVERI (OCIF3)
181

241
KRQHSSQEQT FQL LKLWKHQNKDQDIVKKIIQDIDL CENS VQRHIGHANLT FEQLRSLME (OCIF1)

KRQHSSQEQT FQL LKLWKHQNKDQDIVKKIIQDIDL CENS VQRHIGHANLS----- (OCIF3)
241

301
SLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALKH SKTYHFPKT (OCIF1)

-----LWRIKNGDQDTLKGLMHALKH SKTYHFPKT (OCIF3)
292

361
VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF1)

VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF3)
322

Fig. 11

1
MNNLLCCALVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF1)
** *****
MNKLLCCSLVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF4)
1
61
VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)

VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF4)
61
121
HRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTQKGNAT (OCIF1)

HRSCPPGFGVVQAGTCQCAAKLIRIMQSQIVVTV (OCIF4)
121

Fig. 12

1
MNNLLCCALVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF1)
** *****
MNKLLCCALVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKT (OCIF5)
1
61
VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)

VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF5)
61
121
HRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTQKGNAT (OCIF1)
***** *
HRSCPPGFGVVQAGCRRRPKPQICI (OCIF5)
121

Fig. 13

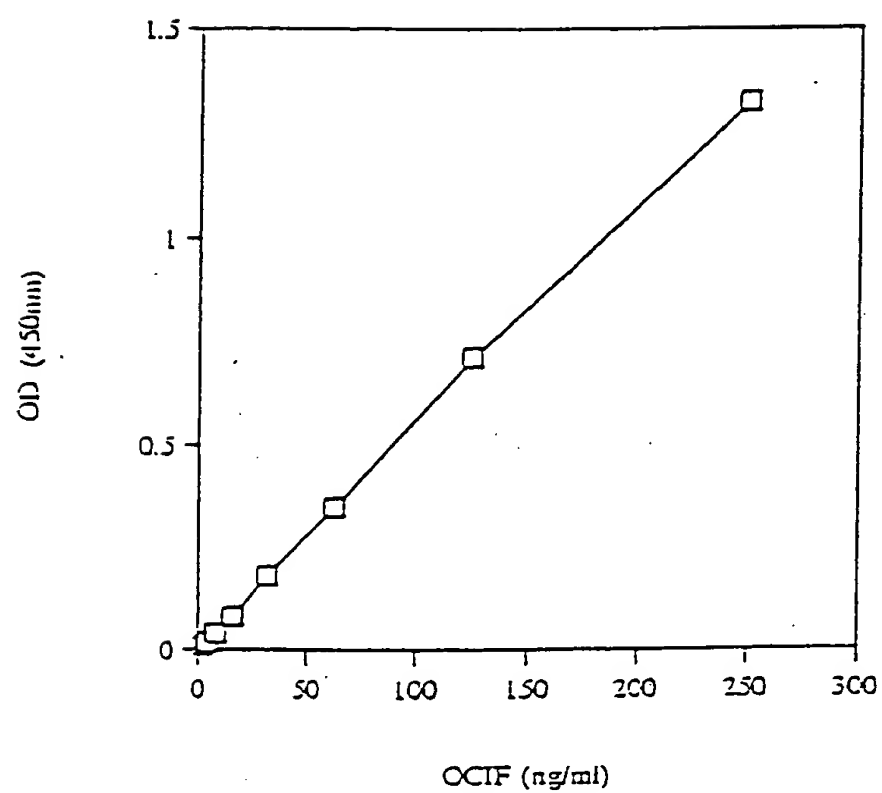


Fig. 14

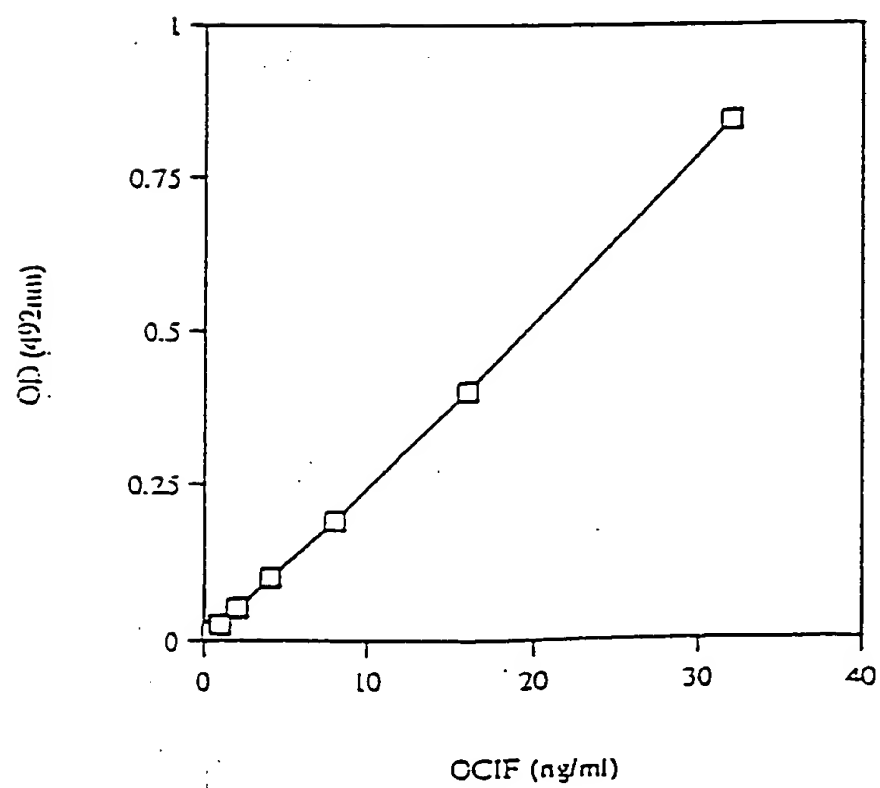
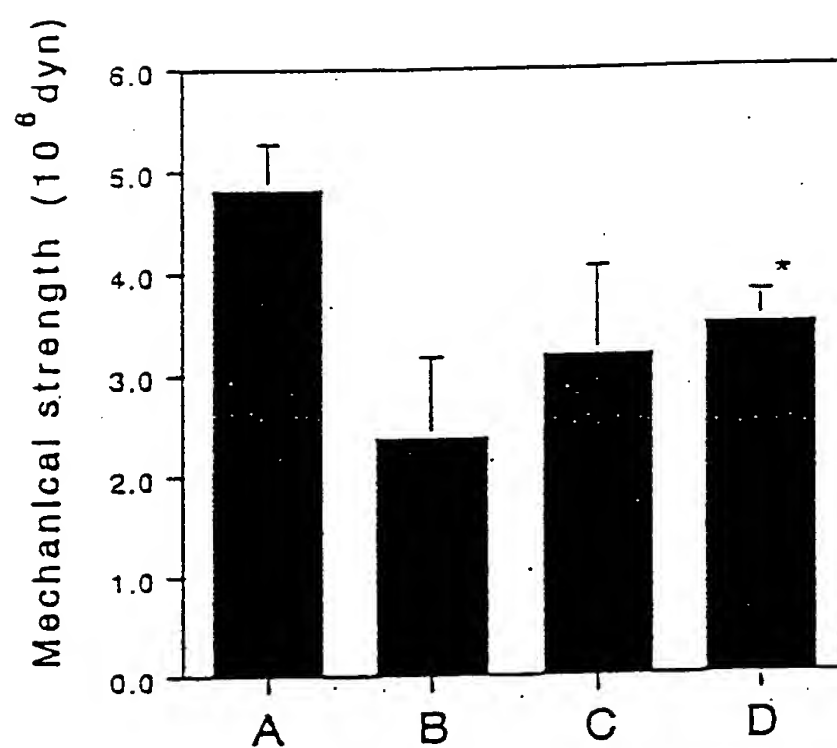


Fig. 15



A : Normal rat

B : Denervated rat + Vehicle

C : Denervated rat + OCIF 10 μ g/kg/day

D : Denervated rat + OCIF 100 μ g/kg/day

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: SNOW BRANDS MILK PRODUCTS CO., LTD.

(B) STREET:

(C) CITY:

(D) STATE:

(E) COUNTRY:

(F) POSTAL CODE (ZIP):

(G) TELEPHONE:

(H) TELEFAX:

(I) TELEX:

(ii) TITLE OF INVENTION: Novel proteins and methods for producing the
proteins

(iii) NUMBER OF SEQUENCES: 105

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER:

(C) OPERATING SYSTEM:

(D) SOFTWARE: Wordperfect windows

(v) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: JP

(B) FILE REFERENCE:

(C) FILING DATE:

(2) INFORMATION FOR SEQUENCE ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 6

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide (an internal amino acid sequence of the protein)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 1:

Xaa Tyr His Phe Pro Lys

1

5

(2) INFORMATION FOR SEQUENCE ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 14

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide (an internal amino acid sequence of the protein)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO:2:

Xaa Gln His Ser Xaa Gln Glu Gln Thr Phe Gln Leu Xaa Lys

1

5

10

(2) INFORMATION FOR SEQUENCE ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 12

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide (an internal amino acid sequence of the protein)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 3:

Xaa Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys

1

5

10

(2) INFORMATION FOR SEQUENCE ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 380

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF protein without signal peptide)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO:4:

Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His	Tyr	Asp	Glu	Glu	Thr	Ser
1				5					10					15
His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro	Pro	Gly	Thr	Tyr	Leu	Lys
				20					25					30
Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr	Val	Cys	Ala	Pro	Cys	Pro
				35					40					45
Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His	Thr	Ser	Asp	Glu	Cys	Leu
				50					55					60
Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu	Gln	Tyr	Val	Lys	Gln	Glu
				65					70					75
Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys	Glu	Cys	Lys	Glu	Gly	Arg
				80					85					90
Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys	His	Arg	Ser	Cys	Pro	Pro
				95					100					105
Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr	Pro	Glu	Arg	Asn	Thr	Val
				110					115					120
Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe	Ser	Asn	Glu	Thr	Ser	Ser
				125					130					135
Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn	Cys	Ser	Val	Phe	Gly	Leu
				140					145					150
Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr	His	Asp	Asn	Ile	Cys	Ser
				155					160					165
Gly	Asn	Ser	Glu	Ser	Thr	Gln	Lys	Cys	Gly	Ile	Asp	Val	Thr	Leu
				170					175					180
Cys	Glu	Glu	Ala	Phe	Phe	Arg	Phe	Ala	Val	Pro	Thr	Lys	Phe	Thr
				185					190					195
Pro	Asn	Trp	Leu	Ser	Val	Leu	Val	Asp	Asn	Leu	Pro	Gly	Thr	Lys
				200					205					210
Val	Asn	Ala	Glu	Ser	Val	Glu	Arg	Ile	Lys	Arg	Gln	His	Ser	Ser
				215					220					225
Gln	Glu	Gln	Thr	Phe	Gln	Leu	Leu	Lys	Leu	Trp	Lys	His	Gln	Asn
				230					235					240

Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu		
	245	250 255
Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr		
	260	265 270
Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys		
	275	280 285
Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro		
	290	295 300
Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn		
	305	310 315
Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His		
	320	325 330
Ser Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys		
	335	340 345
Lys Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr		
	350	355 360
Gln Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val		
	365	370 375
Lys Ile Ser Cys Leu		
	380	

(2) INFORMATION FOR SEQUENCE ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 401

(B) TYPE : amino acid

(D) TOPOLOGY : linear.

(ii) MOLECULE TYPE : protein (OCIF protein with signal peptide)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 5:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25	30	35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		

40	45	50
Thr Ser Asp Glu Cys	Leu Tyr Cys Ser Pro	Val Cys Lys Glu Leu
55	60	65
Gln Tyr Val Lys Gln	Glu Cys Asn Arg Thr	His Asn Arg Val Cys
70	75	80
Glu Cys Lys Glu Gly	Arg Tyr Leu Glu Ile	Glu Phe Cys Leu Lys
85	90	95
His Arg Ser Cys Pro	Pro Gly Phe Gly Val	Val Gln Ala Gly Thr
100	105	110
Pro Glu Arg Asn Thr	Val Cys Lys Arg Cys	Pro Asp Gly Phe Phe
115	120	125
Ser Asn Glu Thr Ser	Ser Lys Ala Pro Cys	Arg Lys His Thr Asn
130	135	140
Cys Ser Val Phe Gly	Leu Leu Leu Thr Gln	Lys Gly Asn Ala Thr
145	150	155
His Asp Asn Ile Cys	Ser Gly Asn Ser Glu	Ser Thr Gln Lys Cys
160	165	170
Gly Ile Asp Val Thr	Leu Cys Glu Glu Ala	Phe Phe Arg Phe Ala
175	180	185
Val Pro Thr Lys Phe	Thr Pro Asn Trp Leu	Ser Val Leu Val Asp
190	195	200
Asn Leu Pro Gly Thr	Lys Val Asn Ala Glu	Ser Val Glu Arg Ile
205	210	215
Lys Arg Gln His Ser	Ser Gln Glu Gln Thr	Phe Gln Leu Leu Lys
220	225	230
Leu Trp Lys His Gln	Asn Lys Asp Gln Asp	Ile Val Lys Lys Ile
235	240	245
Ile Gln Asp Ile Asp	Leu Cys Glu Asn Ser	Val Gln Arg His Ile
250	255	260
Gly His Ala Asn Leu	Thr Phe Glu Gln Leu	Arg Ser Leu Met Glu
265	270	275
Ser Leu Pro Gly Lys	Lys Val Gly Ala Glu	Asp Ile Glu Lys Thr
280	285	290
Ile Lys Ala Cys Lys	Pro Ser Asp Gln Ile	Leu Lys Leu Leu Ser
295	300	305
Leu Trp Arg Ile Lys	Asn Gly Asp Gln Asp	Thr Leu Lys Gly Leu

310	315	320
Met His Ala Leu Lys	His Ser Lys Thr Tyr	His Phe Pro Lys Thr
325	330	335
Val Thr Gln Ser Leu Lys	Lys Thr Ile Arg	Phe Leu His Ser Phe
340	345	350
Thr Met Tyr Lys Leu Tyr	Gln Lys Leu Phe	Leu Glu Met Ile Gly
355	360	365
Asn Gln Val Gln Ser Val	Lys Ile Ser Cys	Leu
370	375	380

(2) INFORMATION FOR SEQUENCE ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1206

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 6:

```

ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020

```

ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080
 GTCACCTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
 TATCAGAAGT TATTTTGTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200
 TTATAA 1206

(2) INFORMATION FOR SEQUENCE ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 15

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide (a N-terminal amino acid sequence of the protein)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO:7:

Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His	Tyr	Asp	Glu	Glu	Thr	Ser
1			5				10						15	

(2) INFORMATION FOR SEQUENCE NO ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1185

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO:8

ATGAACAAC TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
 CTATACTGCA GCCCCGTGTG CAAGGAGTGC AATCGCACCC ACAACCGCGT GTGCGAATGC 300
 AAGGAAGGGC GCTACCTTGA GATAGAGTTC TGCTTGAAAC ATAGGAGCTG CCCTCCTGGA 360
 TTTGGAGTGG TGCAAGCTGG AACCCAGAG CGAAATACAG TTTGCAAAAG ATGTCCAGAT 420
 GGGTTCTTCT CAAATGAGAC GTCATCTAAA GCACCCTGTA GAAAACACAC AAATTGCAGT 480
 GTCTTTGGTC TCCTGCTAAC TCAGAAAGGA AATGCAACAC ACGACAACAT ATGTTCCGGA 540
 AACAGTGAAT CAACTCAAAA ATGTGGAATA GATGTTACCC TGTGTGAGGA GGCATTCTTC 600
 AGGTTTGCTG TTCCTACAAA GTTTACGCCT AACTGGCTTA GTGTCTTGGT AGACAATTTC 660
 CCTGGCACCA AAGTAAACGC AGAGAGTGTA GAGAGGATAA AACGGCAACA CAGCTCACAA 720

GAACAGACTT TCCAGCTGCT GAAGTTATGG AAACATCAAA ACAAAGACCA AGATATAGTC 780
 AAGAAGATCA TCCAAGATAT TGACCTCTGT GAAAACAGCG TGCAGCGGCA CATTGGACAT 840
 GCTAACCTCA CCTTCGAGCA GCTTCGTAGC TTGATGGAAA GCTTACCGGG AAAGAAAGTG 900
 GGAGCAGAAG ACATTGAAAA AACAATAAAG GCATGCAAAC CCAGTGACCA GATCCTGAAG 960
 CTGCTCAGTT TGTGGCGAAT AAAAAATGGC GACCAAGACA CCTTGAAGGG CCTAATGCAC 1020
 GCACTAAAGC ACTCAAAGAC GTACCACTTT CCCAAAAGTG TCACTCAGAG TCTAAAGAAG 1080
 ACCATCAGGT TCCTTCACAG CTTTACAATG TACAAATTGT ATCAGAAGTT ATTTTATAGAA 1140
 ATGATAGGTA ACCAGGTCCA ATCAGTAAAA ATAAGCTGCT TATAA 1185

(2) INFORMATION FOR SEQUENCE ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 394

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF2)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
 -20 -15 -10
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
 -5 -1 1 5
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
 10 15 20
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
 25 30 35
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
 40 45 50
 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Cys
 55 60 65
 Asn Arg Thr His Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr
 70 75 80
 Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly
 85 90 95
 Phe Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys
 100 105 110
 Lys Arg Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys
 115 120 125

Ala Pro Cys Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu		
130	135	140
Leu Thr Gln Lys Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly		
145	150	155
Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile Asp Val Thr Leu Cys		
160	165	170
Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr Lys Phe Thr Pro		
175	180	185
Asn Trp Leu Ser Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val		
190	195	200
Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln		
205	210	215
Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys		
220	225	230
Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys		
235	240	245
Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe		
250	255	260
Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val		
265	270	275
Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser		
280	285	290
Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly		
295	300	305
Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser		
310	315	320
Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys		
325	330	335
Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln		
340	345	350
Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys		
355	360	365
Ile Ser Cys Leu		
370	373	

(2) INFORMATION FOR SEQUENCE ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1089
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF3)

(xi) SEQUENCE DESCRIPTION ID NO: 10:

```
ATGAACAAGT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC AGTTTGTGGC GAATAAAAAA TGGCGACCAA 900
GACACCTTGA AGGGCCTAAT GCACGCACTA AAGCACTCAA AGACGTACCA CTTTCCCAAA 960
ACTGTCACTC AGAGTCTAAA GAAGACCATC AGGTTCTTTC ACAGCTTCAC AATGTACAAA 1020
TTGTATCAGA AGTTATTTTT AGAAATGATA GGTAACCAGG TCCAATCAGT AAAAATAAGC 1080
TGCTTATAA 1089
```

(2) INFORMATION FOR SEQUENCE ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 362
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Asn Lys Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25	30	35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
40	45	50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
55	60	65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
70	75	80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
85	90	95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		

250	255	260
Gly His Ala Asn Leu Ser Leu Trp Arg Ile	Lys Asn Gly Asp Gln	
265	270	275
Asp Thr Leu Lys Gly Leu Met His Ala Leu	Lys His Ser Lys Thr	
280	285	290
Tyr His Phe Pro Lys Thr Val Thr Gln Ser	Leu Lys Lys Thr Ile	
295	300	305
Arg Phe Leu His Ser Phe Thr Met Tyr Lys	Leu Tyr Gln Lys Leu	
310	315	320
Phe Leu Glu Met Ile Gly Asn Gln Val Gln	Ser Val Lys Ile Ser	
325	330	335
Cys Leu		
340	341	

(2) INFORMATION FOR SEQUENCE ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 465
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF4)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 12:

ATGAACAAGT TGCTGTGCTG CTCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC	60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG	120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC	180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT	240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC	300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA	360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GTACGTGTCA ATGTGCAGCA	420
AAATTAATTA GGATCATGCA AAGTCAGATA GTTGTGACAG TTTAG	465

(2) INFORMATION FOR SEQUENCE ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 154
- (B) TYPE : amino acid

(C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : protein (OCIF4)
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Met	Asn	Lys	Leu	Leu	Cys	Cys	Ser	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15						-0			
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1					5			
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105					110				
Cys	Gln	Cys	Ala	Ala	Lys	Leu	Ile	Arg	Ile	Met	Gln	Ser	Gln	Ile
115					120					125				
Val	Val	Thr	Val											
130					133									

(2) INFORMATION FOR SEQUENCE ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 438
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF5)

(xi) SEQUENCE DESCRIPTION ID NO: 14:

ATGAACAAGT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60

CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AACCTCTCA TCAGCTGTTG 120
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GATGCAGGAG AAGACCCAAG 420
 CCACAGATAT GTATCTGA 438

(2) INFORMATION FOR SEQUENCE ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH :140

(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF5)

(xi) SEQUENCE DESCRIPTION: ID NO: 15:

Met Asn Lys Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
 -20 -15 -10
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
 -5 -1 1 5
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
 10 15 20
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
 25 30 35
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
 40 45 50
 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
 55 60 65
 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
 70 75 80
 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
 85 90 95
 His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Cys
 100 105 110
 Arg Arg Arg Pro Lys Pro Gln Ile Cys Ile
 115 120 124

(2) INFORMATION FOR SEQUENCE ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer T3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

AATTAACCCT CACTAAAGGG

20

(2) INFORMATION FOR SEQUENCE ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 22

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer T7)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GTAATACGAC TCACTATAGG GC

22

(2) INFORMATION FOR SEQUENCE ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 18:

ACATCAAAAC AAAGACCAAG

20

(2) INFORMATION FOR SEQUENCE ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF2)
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 19:
TCTTGGTCTT TGTTTTGATG

20

(2) INFORMATION FOR SEQUENCE ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF3)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 20:

TTATTCGCCA CAAACTGAGC

20

(2) INFORMATION FOR SEQUENCE ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF4)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 21:

TTGTGAAGCT GTGAAGGAAC

20

(2) INFORMATION FOR SEQUENCE ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF5)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 22:

GCTCAGTTTG TGGCGAATAA

20

(2) INFORMATION FOR SEQUENCE ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF6)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 23:

GTGGGAGCAG AAGACATTGA

20

(2) INFORMATION FOR SEQUENCE ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF7)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 24:

AATGAACAAC TTGCTGTGCT

20

(2) INFORMATION FOR SEQUENCE ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF8)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 25:

TGACAAATGT CCTCCTGGTA

20

(2) INFORMATION FOR SEQUENCE ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF9)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 26:
AGGTAGGTAC CAGGAGGACA

20

(2) INFORMATION FOR SEQUENCE ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF10)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 27:

GAGCTGCCCT CCTGGATTG

20

(2) INFORMATION FOR SEQUENCE ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF11)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 28:

CAAACGTAT TTCGCTCTGG

20

(2) INFORMATION FOR SEQUENCE ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF12)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 29:

GTGTGAGGAG GCATTCTCA

20

(2) INFORMATION FOR SEQUENCE ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 32

(B) TYPE : nucleic acid
 (C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer C19SF)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 30:
 GAATCAACTC AAAAAAGTGG AATAGATGTT AC

32

(2) INFORMATION FOR SEQUENCE ID NO: 31:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH : 32
 (B) TYPE : nucleic acid
 (C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer C19SR)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 31:
 GTAACATCTA TTCCAATTTT TTGAGTTGAT TC

32

(2) INFORMATION FOR SEQUENCE ID NO: 32:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH : 30
 (B) TYPE : nucleic acid
 (C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer C20SF)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 32:

ATAGATGTTA CCCTGAGTGA GGAGGCATTC

30

(2) INFORMATION FOR SEQUENCE ID NO: 33:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH : 30
 (B) TYPE : nucleic acid
 (C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer C20SR)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 33:

GAATGCCTCC TCACTCAGGG TAACATCTAT

(2) INFORMATION FOR SEQUENCE ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 31

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer C21SF)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 34:

CAAGATATTG ACCTCAGTGA AAACAGCGTG C

31

(2) INFORMATION FOR SEQUENCE ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 31

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer C21SR)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 35:

GCACGCTGTT TCACTGAGG GCAATATCTT G

31

(2) INFORMATION FOR SEQUENCE ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 31

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer C22SF)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 36:

AAAACAATAA AGGCAAGCAA ACCCAGTGAC C

31

(2) INFORMATION FOR SEQUENCE ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 31

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer C22SR)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 37:
 GGTCAGTGGG TTGCTTGCC TTTATTGTTT T 31

(2) INFORMATION FOR SEQUENCE ID NO: 38:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH : 31
 (B) TYPE : nucleic acid
 (C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer C23SF)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 38:
 TCAGTAAAAA TAAGCAGCTT ATAAGTGGCC A 31

(2) INFORMATION FOR SEQUENCE ID NO: 39:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH : 31
 (B) TYPE : nucleic acid
 (C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer C23SR)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 39:
 TGGCCAGTTA TAAGCTGCTT ATTTTACTG A 31

(2) INFORMATION FOR SEQUENCE ID NO: 40:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH : 22
 (B) TYPE : nucleic acid
 (C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer IF 14)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 40:
 TTGGGGTTTA TTGGAGGAGA TG 22

(2) INFORMATION FOR SEQUENCE ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DCR1F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 41:

ACCACCCAGG AACCTTGCCC TGACCACTAC TACACA

36

(2) INFORMATION FOR SEQUENCE ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DCR1R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 42:

GTCAGGGCAA GGTTCCTGGG TGGTCCACTT AATGGA

36

(2) INFORMATION FOR SEQUENCE ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DCR2F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 43:

ACCGTGTGCG CCGAATGCAA GGAAGGGCGC TACCTT

36

(2) INFORMATION FOR SEQUENCE ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DCR2R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 44:

TTCCTTG CAT TCGGCGCACA CGGTCTTCCA CTTTGC

36

(2) INFORMATION FOR SEQUENCE ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DCR3F)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 45:

AACCGCGTGT GCAGATGTCC AGATGGGTTC TTCTCA

36

(2) INFORMATION FOR SEQUENCE ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DCR3R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 46:

ATCTGGACAT CTGCACACGC GGTGTGGGT GCGATT

36

(2) INFORMATION FOR SEQUENCE ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DCR4F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 47:

ACAGTTTGCA AATCCGAAA CAGTGAATCA ACTCAA

36

(2) INFORMATION FOR SEQUENCE ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DCR4R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 48:

ACTGTTTCCG GATTGCAAA CTGTATTTCG CTCTGG

36

(2) INFORMATION FOR SEQUENCE ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DDD1F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 49:

AATGTGGAAT AGATATTGAC CTCTGTGAAA ACAGCG

36

(2) INFORMATION FOR SEQUENCE ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DDD1R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 50:

AGAGGTCAAT ATCTATTCCA CATTTTGTGAG TTGATT

36

(2) INFORMATION FOR SEQUENCE ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DDD2F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 51:

AGATCATCCA AGACGCACTA AAGCACTCAA AGACGT

36

(2) INFORMATION FOR SEQUENCE ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DDD2R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 52:

GCTTTAGTGC GTCTTGATG ATCTTCTTGA CTATAT

36

(2) INFORMATION FOR SEQUENCE ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 29

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer XhoI F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 53:

GGCTCGAGCG CCCAGCCGCC GCCTCCAAG

29

(2) INFORMATION FOR SEQUENCE ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF 16)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 54:

TTTGAGTGCT TTAGTGCGTG

20

(2) INFORMATION FOR SEQUENCE ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 30

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer CL F)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 55:
 TCAGTAAAAA TAAGCTAACT GGAAATGGCC 30

(2) INFORMATION FOR SEQUENCE ID NO: 56:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH : 30
 (B) TYPE : nucleic acid
 (C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer CL R)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 56:
 GGCCATTTC AGTTAGCTTA TTTTACTGA 30

(2) INFORMATION FOR SEQUENCE ID NO: 57:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH : 29
 (B) TYPE : nucleic acid
 (C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer CC R)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 57:
 CCGGATCCTC AGTGCTTTAG TCGTGTCAT 29

(2) INFORMATION FOR SEQUENCE ID NO: 58:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH : 29
 (B) TYPE : nucleic acid
 (C) STRANDEDNESS : single
 (D) TOPOLOGY : linear
 (ii) MOLECULE TYPE : synthetic DNA (primer CCD2 R)
 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 58:
 CCGGATCCTC ATTGGATGAT CTTCTTGAC 29

(2) INFORMATION FOR SEQUENCE ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 29

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer CCD1 R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 59:

CCGGATCCTC ATATTCCACA TTTTGTGAGT

29

(2) INFORMATION FOR SEQUENCE ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 29

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer CCR4 R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 60:

CCGGATCCTC ATTTGCAAAC TGTATTTTCG

29

(2) INFORMATION FOR SEQUENCE ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 29

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer CCR3 R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 61:

CCGGATCCTC ATTCGCACAC GCGGTTGTG

29

(2) INFORMATION FOR SEQUENCE ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 401

(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-C19S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 62:

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15					-10				
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1				5				
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105					110				
Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
115					120					125				
Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
130					135					140				
Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr
145					150					155				
His	Asp	Asn	Ile	Cys	Ser	Gly	Asn	Ser	Glu	Ser	Thr	Gln	Lys	Ser
160					165					170				
Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala	Phe	Phe	Arg	Phe	Ala
175					180					185				
Val	Pro	Thr	Lys	Phe	Thr	Pro	Asn	Trp	Leu	Ser	Val	Leu	Val	Asp
190					195					200				
Asn	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu	Ser	Val	Glu	Arg	Ile
205					210					215				
Lys	Arg	Gln	His	Ser	Ser	Gln	Glu	Gln	Thr	Phe	Gln	Leu	Leu	Lys
220					225					230				

Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
340	345	350
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
355	360	365
Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
370	375	380

(2) INFORMATION FOR SEQUENCE ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 401
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-C20S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 63:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		

25	30	35
Val Cys Ala Pro Cys	Pro Asp His Tyr Tyr	Thr Asp Ser Trp His
40	45	50
Thr Ser Asp Glu Cys	Leu Tyr Cys Ser Pro	Val Cys Lys Glu Leu
55	60	65
Gln Tyr Val Lys Gln	Glu Cys Asn Arg Thr	His Asn Arg Val Cys
70	75	80
Glu Cys Lys Glu Gly	Arg Tyr Leu Glu Ile	Glu Phe Cys Leu Lys
85	90	95
His Arg Ser Cys Pro	Pro Gly Phe Gly Val	Val Gln Ala Gly Thr
100	105	110
Pro Glu Arg Asn Thr	Val Cys Lys Arg Cys	Pro Asp Gly Phe Phe
115	120	125
Ser Asn Glu Thr Ser	Ser Lys Ala Pro Cys	Arg Lys His Thr Asn
130	135	140
Cys Ser Val Phe Gly	Leu Leu Leu Thr Gln	Lys Gly Asn Ala Thr
145	150	155
His Asp Asn Ile Cys	Ser Gly Asn Ser Glu	Ser Thr Gln Lys Cys
160	165	170
Gly Ile Asp Val Thr	Leu Ser Glu Glu Ala	Phe Phe Arg Phe Ala
175	180	185
Val Pro Thr Lys Phe	Thr Pro Asn Trp Leu	Ser Val Leu Val Asp
190	195	200
Asn Leu Pro Gly Thr	Lys Val Asn Ala Glu	Ser Val Glu Arg Ile
205	210	215
Lys Arg Gln His Ser	Ser Gln Glu Gln Thr	Phe Gln Leu Leu Lys
220	225	230
Leu Trp Lys His Gln	Asn Lys Asp Gln Asp	Ile Val Lys Lys Ile
235	240	245
Ile Gln Asp Ile Asp	Leu Cys Glu Asn Ser	Val Gln Arg His Ile
250	255	260
Gly His Ala Asn Leu	Thr Phe Glu Gln Leu	Arg Ser Leu Met Glu
265	270	275
Ser Leu Pro Gly Lys	Lys Val Gly Ala Glu	Asp Ile Glu Lys Thr
280	285	290
Ile Lys Ala Cys Lys	Pro Ser Asp Gln Ile	Leu Lys Leu Leu Ser

295	300	305
Leu Trp Arg Ile Lys	Asn Gly Asp Gln Asp	Thr Leu Lys Gly Leu
310	315	320
Met His Ala Leu Lys	His Ser Lys Thr Tyr	His Phe Pro Lys Thr
325	330	335
Val Thr Gln Ser Leu	Lys Lys Thr Ile Arg	Phe Leu His Ser Phe
340	345	350
Thr Met Tyr Lys Leu	Tyr Gln Lys Leu Phe	Leu Glu Met Ile Gly
355	360	365
Asn Gln Val Gln Ser	Val Lys Ile Ser Cys	Leu
370	375	380

(2) INFORMATION FOR SEQUENCE ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 401
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-C21S)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 64:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser	
-20	-15 -10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His	
-5	-1 1 5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro	
10	15 20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr	
25	30 35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His	
40	45 50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu	
55	60 65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys	
70	75 80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys	
85	90 95

His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Ser Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
340	345	350
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
355	360	365

Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
 370 375 380

(2) INFORMATION FOR SEQUENCE ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 401
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-C22S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 65:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
 -20 -15 -10
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
 -5 -1 1 5
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
 10 15 20
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
 25 30 35
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
 40 45 50
 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
 55 60 65
 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
 70 75 80
 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
 85 90 95
 His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr
 100 105 110
 Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe
 115 120 125
 Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
 130 135 140
 Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr
 145 150 155
 His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys

160	165	170
Gly Ile Asp Val Thr	Leu Cys Glu Glu Ala Phe	Phe Arg Phe Ala
175	180	185
Val Pro Thr Lys Phe	Thr Pro Asn Trp Leu	Ser Val Leu Val Asp
190	195	200
Asn Leu Pro Gly Thr	Lys Val Asn Ala Glu	Ser Val Glu Arg Ile
205	210	215
Lys Arg Gln His Ser	Ser Gln Glu Gln Thr	Phe Gln Leu Leu Lys
220	225	230
Leu Trp Lys His Gln	Asn Lys Asp Gln Asp	Ile Val Lys Lys Ile
235	240	245
Ile Gln Asp Ile Asp	Leu Cys Glu Asn Ser	Val Gln Arg His Ile
250	255	260
Gly His Ala Asn Leu	Thr Phe Glu Gln Leu	Arg Ser Leu Met Glu
265	270	275
Ser Leu Pro Gly Lys	Lys Val Gly Ala Glu	Asp Ile Glu Lys Thr
280	285	290
Ile Lys Ala Ser Lys	Pro Ser Asp Gln Ile	Leu Lys Leu Leu Ser
295	300	305
Leu Trp Arg Ile Lys	Asn Gly Asp Gln Asp	Thr Leu Lys Gly Leu
310	315	320
Met His Ala Leu Lys	His Ser Lys Thr Tyr	His Phe Pro Lys Thr
325	330	335
Val Thr Gln Ser Leu	Lys Lys Thr Ile Arg	Phe Leu His Ser Phe
340	345	350
Thr Met Tyr Lys Leu	Tyr Gln Lys Leu Phe	Leu Glu Met Ile Gly
355	360	365
Asn Gln Val Gln Ser	Val Lys Ile Ser Cys	Leu
370	375	380

(2) INFORMATION FOR SEQUENCE ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 401
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-C23S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 66:

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15						-10			
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1					5			
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105					110				
Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
115					120					125				
Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
130					135					140				
Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr
145					150					155				
His	Asp	Asn	Ile	Cys	Ser	Gly	Asn	Ser	Glu	Ser	Thr	Gln	Lys	Cys
160					165					170				
Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala	Phe	Phe	Arg	Phe	Ala
175					180					185				
Val	Pro	Thr	Lys	Phe	Thr	Pro	Asn	Trp	Leu	Ser	Val	Leu	Val	Asp
190					195					200				
Asn	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu	Ser	Val	Glu	Arg	Ile
205					210					215				
Lys	Arg	Gln	His	Ser	Ser	Gln	Glu	Gln	Thr	Phe	Gln	Leu	Leu	Lys
220					225					230				

Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
340	345	350
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
355	360	365
Asn Gln Val Gln Ser Val Lys Ile Ser Ser Leu		
370	375	380

(2) INFORMATION FOR SEQUENCE ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 360
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-DCR1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 67:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Pro Cys Pro Asp His Tyr Tyr Thr		
-5	-1 1	5
Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val		
10	15	20
Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His		

25	30	35
Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu		
40	45	50
Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val		
55	60	65
Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro		
70	75	80
Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg		
85	90	95
Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys		
100	105	110
Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser		
115	120	125
Thr Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe		
130	135	140
Phe Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser		
145	150	155
Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser		
160	165	170
Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe		
175	180	185
Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile		
190	195	200
Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val		
205	210	215
Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg		
220	225	230
Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp		
235	240	245
Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu		
250	255	260
Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr		
265	270	275
Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His		
280	285	290
Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe		

295	300	305
Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu		
310	315	320
Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
325	330	335

(2) INFORMATION FOR SEQUENCE ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 359
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-DCR2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 68:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25	30	35
Val Cys Ala Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe		
40	45	50
Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln		
55	60	65
Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp		
70	75	80
Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys		
85	90	95
His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly		
100	105	110
Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr		
115	120	125
Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe		
130	135	140

Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val		
145	150	155
Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val		
160	165	170
Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln		
175	180	185
Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val		
190	195	200
Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln		
205	210	215
Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser		
220	225	230
Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile		
235	240	245
Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys		
250	255	260
Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu		
265	270	275
Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe		
280	285	290
Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu		
295	300	305
His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu		
310	315	320
Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
325	330	335

(2) INFORMATION FOR SEQUENCE ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 363

(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-DCR3)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 69:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

-20	-15	-10
Ile Lys Trp Thr Thr	Gln Glu Thr Phe Pro	Pro Lys Tyr Leu His
-5	-1 1	5
Tyr Asp Glu Glu Thr	Ser His Gln Leu Leu	Cys Asp Lys Cys Pro
10	15	20
Pro Gly Thr Tyr Leu	Lys Gln His Cys Thr	Ala Lys Trp Lys Thr
25	30	35
Val Cys Ala Pro Cys	Pro Asp His Tyr Tyr	Thr Asp Ser Trp His
40	45	50
Thr Ser Asp Glu Cys	Leu Tyr Cys Ser Pro	Val Cys Lys Glu Leu
55	60	65
Gln Tyr Val Lys Gln	Glu Cys Asn Arg Thr	His Asn Arg Val Cys
70	75	80
Arg Cys Pro Asp Gly	Phe Phe Ser Asn Glu	Thr Ser Ser Lys Ala
85	90	95
Pro Cys Arg Lys His	Thr Asn Cys Ser Val	Phe Gly Leu Leu Leu
100	105	110
Thr Gln Lys Gly Asn	Ala Thr His Asp Asn	Ile Cys Ser Gly Asn
115	120	125
Ser Glu Ser Thr Gln	Lys Cys Gly Ile Asp	Val Thr Leu Cys Glu
130	135	140
Glu Ala Phe Phe Arg	Phe Ala Val Pro Thr	Lys Phe Thr Pro Asn
145	150	155
Trp Leu Ser Val Leu	Val Asp Asn Leu Pro	Gly Thr Lys Val Asn
160	165	170
Ala Glu Ser Val Glu	Arg Ile Lys Arg Gln	His Ser Ser Gln Glu
175	180	185
Gln Thr Phe Gln Leu	Leu Lys Leu Trp Lys	His Gln Asn Lys Asp
190	195	200
Gln Asp Ile Val Lys	Lys Ile Ile Gln Asp	Ile Asp Leu Cys Glu
205	210	215
Asn Ser Val Gln Arg	His Ile Gly His Ala	Asn Leu Thr Phe Glu
220	225	230
Gln Leu Arg Ser Leu	Met Glu Ser Leu Pro	Gly Lys Lys Val Gly
235	240	245
Ala Glu Asp Ile Glu	Lys Thr Ile Lys Ala	Cys Lys Pro Ser Asp

250	255	260
Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg	Ile Lys Asn Gly Asp	
265	270	275
Gln Asp Thr Leu Lys Gly Leu Met His Ala	Leu Lys His Ser Lys	
280	285	290
Thr Tyr His Phe Pro Lys Thr Val Thr Gln	Ser Leu Lys Lys Thr	
295	300	305
Ile Arg Phe Leu His Ser Phe Thr Met Tyr	Lys Leu Tyr Gln Lys	
310	315	320
Leu Phe Leu Glu Met Ile Gly Asn Gln Val	Gln Ser Val Lys Ile	
325	330	335
Ser Cys Leu		
340		

(2) INFORMATION FOR SEQUENCE ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 359

(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-DCR4)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 70:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser	
-20	-15 -10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His	
-5	-1 1 5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro	
10	15 20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr	
25	30 35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His	
40	45 50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu	
55	60 65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys	
70	75 80

Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
85	90	95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Ser Gly Asn Ser Glu Ser Thr		
115	120	125
Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe		
130	135	140
Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val		
145	150	155
Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val		
160	165	170
Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln		
175	180	185
Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val		
190	195	200
Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln		
205	210	215
Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser		
220	225	230
Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile		
235	240	245
Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys		
250	255	260
Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu		
265	270	275
Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe		
280	285	290
Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu		
295	300	305
His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu		
310	315	320
Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
325	330	335

(2) INFORMATION FOR SEQUENCE ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 326

(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-DDD1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 71:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
-20 -15 -10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
-5 -1 1 5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
10 15 20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
25 30 35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
40 45 50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
55 60 65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
70 75 80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
85 90 95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr
100 105 110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe
115 120 125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130 135 140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr
145 150 155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys
160 165 170
Gly Ile Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile
175 180 185
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu

190	195	200
Ser Leu Pro Gly Lys	Lys Val Gly Ala Glu Asp	Ile Glu Lys Thr
205	210	215
Ile Lys Ala Cys Lys	Pro Ser Asp Gln Ile	Leu Lys Leu Leu Ser
220	225	230
Leu Trp Arg Ile Lys	Asn Gly Asp Gln Asp	Thr Leu Lys Gly Leu
235	240	245
Met His Ala Leu Lys	His Ser Lys Thr Tyr	His Phe Pro Lys Thr
250	255	260
Val Thr Gln Ser Leu	Lys Lys Thr Ile Arg	Phe Leu His Ser Phe
265	270	275
Thr Met Tyr Lys Leu	Tyr Gln Lys Leu Phe	Leu Glu Met Ile Gly
280	285	290
Asn Gln Val Gln Ser	Val Lys Ile Ser Cys	Leu
295	300	305

(2) INFORMATION FOR SEQUENCE ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 327

(B) TYPE: amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-DDD2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 72:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
-20 -15 -10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
-5 -1 1 5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
10 15 20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
25 30 35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
40 45 50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
55 60 65

Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
70	75	80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
85	90	95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys		
250	255	260
Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser		
265	270	275
Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile		
280	285	290
Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
295	300	305

(2) INFORMATION FOR SEQUENCE ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 399

(B) TYPE : amino acid
 (C) STRANDEDNESS : single
 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-CL)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 73:

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20					-15					-10				
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1			5					
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10					15					20				
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25					30					35				
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40					45					50				
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55					60					65				
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70					75					80				
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85					90					95				
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100					105					110				
Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
115					120					125				
Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
130					135					140				
Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr
145					150					155				
His	Asp	Asn	Ile	Cys	Ser	Gly	Asn	Ser	Glu	Ser	Thr	Gln	Lys	Cys
160					165					170				
Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala	Phe	Phe	Arg	Phe	Ala
175					180					185				
Val	Pro	Thr	Lys	Phe	Thr	Pro	Asn	Trp	Leu	Ser	Val	Leu	Val	Asp
190					195					200				
Asn	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu	Ser	Val	Glu	Arg	Ile

205	210	215
Lys Arg Gln His Ser	Ser Gln Glu Gln Thr	Phe Gln Leu Leu Lys
220	225	230
Leu Trp Lys His Gln	Asn Lys Asp Gln Asp	Ile Val Lys Lys Ile
235	240	245
Ile Gln Asp Ile Asp	Leu Cys Glu Asn Ser	Val Gln Arg His Ile
250	255	260
Gly His Ala Asn Leu	Thr Phe Glu Gln Leu	Arg Ser Leu Met Glu
265	270	275
Ser Leu Pro Gly Lys	Lys Val Gly Ala Glu	Asp Ile Glu Lys Thr
280	285	290
Ile Lys Ala Cys Lys	Pro Ser Asp Gln Ile	Leu Lys Leu Leu Ser
295	300	305
Leu Trp Arg Ile Lys	Asn Gly Asp Gln Asp	Thr Leu Lys Gly Leu
310	315	320
Met His Ala Leu Lys	His Ser Lys Thr Tyr	His Phe Pro Lys Thr
325	330	335
Val Thr Gln Ser Leu	Lys Lys Thr Ile Arg	Phe Leu His Ser Phe
340	345	350
Thr Met Tyr Lys Leu	Tyr Gln Lys Leu Phe	Leu Glu Met Ile Gly
355	360	365
Asn Gln Val Gln Ser	Val Lys Ile Ser	
370	375	

(2) INFORMATION FOR SEQUENCE ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 351

(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-CC)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 74:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
-20 -15 -10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
-5 -1 1 5

Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25	30	35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
40	45	50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
55	60	65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
70	75	80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
85	90	95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275

Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr
280 285 290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser
295 300 305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu
310 315 320
Met His Ala Leu Lys His
325 330

(2) INFORMATION FOR SEQUENCE ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 272
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CDD2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 75:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
-20 -15 -10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
-5 -1 1 5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
10 15 20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
25 30 35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
40 45 50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
55 60 65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
70 75 80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
85 90 95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr
100 105 110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe
115 120 125

Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
130						135								140
Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr
145						150								155
His	Asp	Asn	Ile	Cys	Ser	Gly	Asn	Ser	Glu	Ser	Thr	Gln	Lys	Cys
160						165								170
Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala	Phe	Phe	Arg	Phe	Ala
175						180								185
Val	Pro	Thr	Lys	Phe	Thr	Pro	Asn	Trp	Leu	Ser	Val	Leu	Val	Asp
190						195								200
Asn	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu	Ser	Val	Glu	Arg	Ile
205						210								215
Lys	Arg	Gln	His	Ser	Ser	Gln	Glu	Gln	Thr	Phe	Gln	Leu	Leu	Lys
220						225								230
Leu	Trp	Lys	His	Gln	Asn	Lys	Asp	Gln	Asp	Ile	Val	Lys	Lys	Ile
235						240								245
Ile	Gln													
250														

(2) INFORMATION FOR SEQUENCE ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 197
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CDD1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 76:

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20						-15								-10
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5					-1	1					5			
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10						15					20			
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25						30					35			
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His

40	45	50
Thr Ser Asp Glu Cys	Leu Tyr Cys Ser Pro	Val Cys Lys Glu Leu
55	60	65
Gln Tyr Val Lys Gln	Glu Cys Asn Arg Thr	His Asn Arg Val Cys
70	75	80
Glu Cys Lys Glu Gly	Arg Tyr Leu Glu Ile	Glu Phe Cys Leu Lys
85	90	95
His Arg Ser Cys Pro	Pro Gly Phe Gly Val	Val Gln Ala Gly Thr
100	105	110
Pro Glu Arg Asn Thr	Val Cys Lys Arg Cys	Pro Asp Gly Phe Phe
115	120	125
Ser Asn Glu Thr Ser	Ser Lys Ala Pro Cys	Arg Lys His Thr Asn
130	135	140
Cys Ser Val Phe Gly	Leu Leu Leu Thr Gln	Lys Gly Asn Ala Thr
145	150	155
His Asp Asn Ile Cys	Ser Gly Asn Ser Glu	Ser Thr Gln Lys Cys
160	165	170
Gly Ile		
175		

(2) INFORMATION FOR SEQUENCE ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 143
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CCR4)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 77:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25	30	35

Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
 40 45 50
 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
 55 60 65
 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
 70 75 80
 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
 85 90 95
 His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr
 100 105 110
 Pro Glu Arg Asn Thr Val Cys Lys
 115 120

(2) INFORMATION FOR SEQUENCE ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 106

(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CCR3)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 78:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
 -20 -15 -10
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
 -5 -1 1 5
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
 10 15 20
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
 25 30 35
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
 40 45 50
 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
 55 60 65
 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
 70 75 80
 Glu

(2) INFORMATION FOR SEQUENCE ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 393

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CBst)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 79:

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Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
  -20          -15          -10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
  -5          -1   1          5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
10          15          20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
25          30          35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
40          45          50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu
55          60          65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys
70          75          80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
85          90          95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr
100         105         110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe
115         120         125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130         135         140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr
145         150         155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys
160         165         170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala

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175	180	185
Val Pro Thr Lys Phe	Thr Pro Asn Trp Leu	Ser Val Leu Val Asp
190	195	200
Asn Leu Pro Gly Thr	Lys Val Asn Ala Glu	Ser Val Glu Arg Ile
205	210	215
Lys Arg Gln His Ser	Ser Gln Glu Gln Thr	Phe Gln Leu Leu Lys
220	225	230
Leu Trp Lys His Gln	Asn Lys Asp Gln Asp	Ile Val Lys Lys Ile
235	240	245
Ile Gln Asp Ile Asp	Leu Cys Glu Asn Ser	Val Gln Arg His Ile
250	255	260
Gly His Ala Asn Leu	Thr Phe Glu Gln Leu	Arg Ser Leu Met Glu
265	270	275
Ser Leu Pro Gly Lys	Lys Val Gly Ala Glu	Asp Ile Glu Lys Thr
280	285	290
Ile Lys Ala Cys Lys	Pro Ser Asp Gln Ile	Leu Lys Leu Leu Ser
295	300	305
Leu Trp Arg Ile Lys	Asn Gly Asp Gln Asp	Thr Leu Lys Gly Leu
310	315	320
Met His Ala Leu Lys	His Ser Lys Thr Tyr	His Phe Pro Lys Thr
325	330	335
Val Thr Gln Ser Leu	Lys Lys Thr Ile Arg	Phe Leu His Ser Phe
340	345	350
Thr Met Tyr Lys Leu	Tyr Gln Lys Leu Phe	Leu Glu Met Ile Gly
355	360	365
Asn Leu Val		
370		

(2) INFORMATION FOR SEQUENCE ID NO: 80:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 321

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CSph)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 80:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25	30	35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
40	45	50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
55	60	65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
70	75	80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
85	90	95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		

250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Ser Leu Asp		
295	300	

(2) INFORMATION FOR SEQUENCE ID NO: 81:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 202

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CBsp)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 81:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
10	15	20
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
25	30	35
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
40	45	50
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
55	60	65
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
70	75	80
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
85	90	95
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
100	105	110
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
115	120	125
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
130	135	140

Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
145 150 155
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr
160 165 170
His Asp Asn Ile Cys Ser Gly
175 180

(2) INFORMATION FOR SEQUENCE ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 84

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CPst)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 82:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser
-20 -15 -10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His
-5 -1 1 5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro
10 15 20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr
25 30 35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His
40 45 50
Thr Ser Asp Glu Cys Leu Tyr Leu Val
55 60 63

(2) INFORMATION FOR SEQUENCE ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1206

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-C19S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 83:

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ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCGCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AAAGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCTTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080
GTCCTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
TATCAGAAGT TATTTT TAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200
TTATAA 1206

```

(2) INFORMATION FOR SEQUENCE ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1206

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-C20S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 84:

```

ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCGCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300

```


CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAGA GCGAAATACA 420
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
 CTGAGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
 AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAACT 1080
 GTCCTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
 TATCAGAAGT TATTTTTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200
 TTATAA 1206

(2) INFORMATION FOR SEQUENCE ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1206

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-C21S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 85:

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAGA GCGAAATACA 420
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600

CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
 AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCAG TGAAAACAGC 840
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
 TATCAGAAGT TATTTTTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200
 TTATAA 1206

(2) INFORMATION FOR SEQUENCE ID NO: 86:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1206

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-C22S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 86:

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
 AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900

AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCAAGCAAA 960
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080
 GTCACCTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
 TATCAGAAGT TATTTTGTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200
 TTATAA 1206

(2) INFORMATION FOR SEQUENCE ID NO: 87:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1206

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-C23S)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 87:

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600
 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
 AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080
 GTCACCTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
 TATCAGAAGT TATTTTGTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCAGC 1200

(2) INFORMATION FOR SEQUENCE ID NO: 88:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1083

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DCR1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 88:

```

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAACCTT GCCCTGACCA CTACTACACA GACAGCTGGC ACACCAGTGA CGAGTGTCTA 120
TACTGCAGCC CCGTGTGCAA GGAGCTGCAG TACGTCAAGC AGGAGTGCAA TCGCACCCAC 180
AACCGCGTGT GCGAATGCAA GGAAGGGCGC TACCTTGAGA TAGAGTTCTG CTTGAAACAT 240
AGGAGCTGCC CTCCTGGATT TGGAGTGGTG CAAGCTGGAA CCCCAGAGCG AAATACAGTT 300
TGCAAAAGAT GTCCAGATGG GTTCTTCTCA AATGAGACGT CATCTAAAGC ACCCTGTAGA 360
AAACACACAA ATTGCAGTGT CTTTGGTCTC CTGCTAACTC AGAAAGGAAA TGCAACACAC 420
GACAACATAT GTTCCGGAAA CAGTGAATCA ACTCAAAAAT GTGGAATAGA TGTTACCCTG 480
TGTGAGGAGG CATTCTTCAG GTTTGCTGTT CCTACAAAGT TTACGCCTAA CTGGCTTAGT 540
GTCTTGGTAG ACAATTTGCC TGGCACCAAA GTAAACGCAG AGAGTGTAGA GAGGATAAAA 600
CGGCAACACA GCTCACAAGA ACAGACTTTC CAGCTGCTGA AGTTATGGAA ACATCAAAAC 660
AAAGACCAAG ATATAGTCAA GAAGATCATC CAAGATATTG ACCTCTGTGA AAACAGCGTG 720
CAGCGGCACA TTGGACATGC TAACCTCACC TTCGAGCAGC TTCGTAGCTT GATGGAAAGC 780
TTACCGGGAA AGAAAGTGGG AGCAGAAGAC ATTGAAAAAA CAATAAAGGC ATGCAAACCC 840
AGTGACCAGA TCCTGAAGCT GCTCAGTTTG TGGCGAATAA AAAATGGCGA CCAAGACACC 900
TTGAAGGGCC TAATGCACGC ACTAAAGCAC TCAAAGACGT ACCACTTTCC CAAAACGTGTC 960
ACTCAGAGTC TAAAGAAGAC CATCAGGTTC CTTACAGCT TCACAATGTA CAAATTGTAT 1020
CAGAAGTTAT TTTTAGAAAT GATAGGTAAC CAGGTCCAAT CAGTAAAAAT AAGCTGCTTA 1080
TAA 1083

```

(2) INFORMATION FOR SEQUENCE ID NO: 89:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1080

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DCR2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 89:

```
ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCG AATGCAAGGA AGGGCGCTAC CTTGAGATAG AGTTCTGCTT GAAACATAGG 240
AGCTGCCCTC CTGGATTTGG AGTGGTGCAA GCTGGAACCC CAGAGCGAAA TACAGTTTGC 300
AAAAGATGTC CAGATGGGTT CTTCTCAAAT GAGACGTCAT CTAAAGCACC CTGTAGAAAA 360
CACACAAATT GCAGTGTCTT TGGTCTCCTG CTAAGTCAGA AAGGAAATGC AACACACGAC 420
AACATATGTT CCGGAAACAG TGAATCAACT CAAAAATGTG GAATAGATGT TACCCTGTGT 480
GAGGAGGCAT TCTTCAGGTT TGCTGTTTCT ACAAAGTTTA CGCCTAACTG GCTTAGTGTC 540
TTGGTAGACA ATTTGCCTGG CACCAAAGTA AACGCAGAGA GTGTAGAGAG GATAAAACGG 600
CAACACAGCT CACAAGAACA GACTTTCCAG CTGCTGAAGT TATGGAAACA TCAAAACAAA 660
GACCAAGATA TAGTCAAGAA GATCATCCAA GATATTGACC TCTGTGAAAA CAGCGTGCAG 720
CGGCACATTG GACATGCTAA CCTCACCTTC GAGCAGCTTC GTAGCTTGAT GGAAAGCTTA 780
CCGGGAAAGA AAGTGGGAGC AGAAGACATT GAAAAAACA TAAAGGCATG CAAACCCAGT 840
GACCAGATCC TGAAGCTGCT CAGTTTGTGG CGAATAAAAA ATGGCGACCA AGACACCTTG 900
AAGGGCCTAA TGCACGCACT AAAGCACTCA AAGACGTACC ACTTTCCCAA AACTGTCACT 960
CAGAGTCTAA AGAAGACCAT CAGGTTTCTT CACAGCTTCA CAATGTACAA ATTGTATCAG 1020
AAGTTATTTT TAGAAATGAT AGGTAACCAG GTCCAATCAG TAAAAATAAG CTGCTTATAA 1080
```

(2) INFORMATION FOR SEQUENCE ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1092

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DCR3)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 90:

```
ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
```

CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
 CACAACCGCG TGTGCAGATG TCCAGATGGG TTCTTCTCAA ATGAGACGTC ATCTAAAGCA 360
 CCCTGTAGAA AACACACAAA TTGCAGTGTC TTTGGTCTCC TGCTAACTCA GAAAGGAAAT 420
 GCAACACACG ACAACATATG TTCCGGAAAC AGTGAATCAA CTCAAAAATG TGGAATAGAT 480
 GTTACCCTGT GTGAGGAGGC ATTCTTCAGG TTTGCTGTTC CTACAAAGTT TACGCCTAAC 540
 TGGCTTAGTG TCTTGGTAGA CAATTTGCCT GGCACCAAAG TAAACGCAGA GAGTGTAGAG 600
 AGGATAAAAC GGCAACACAG CTCACAAGAA CAGACTTTCC AGCTGCTGAA GTTATGGAAA 660
 CATCAAAACA AAGACCAAGA TATAGTCAAG AAGATCATCC AAGATATTGA CCTCTGTGAA 720
 AACAGCGTGC AGCGGCACAT TGGACATGCT AACCTCACCT TCGAGCAGCT TCGTAGCTTG 780
 ATGGAAAGCT TACCGGGAAA GAAAGTGGGA GCAGAAGACA TTGAAAAAAC AATAAAGGCA 840
 TGCAAACCCA GTGACCAGAT CCTGAAGCTG CTCAGTTTGT GGCGAATAAA AAATGGCGAC 900
 CAAGACACCT TGAAGGGCCT AATGCACGCA CTAAAGCACT CAAAGACGTA CCACTTTCCC 960
 AAAACTGTCA CTCAGAGTCT AAAGAAGACC ATCAGGTTCC TTCACAGCTT CACAATGTAC 1020
 AAATTGTATC AGAAGTTATT TTTAGAAATG ATAGGTAACC AGGTCCAATC AGTAAAAATA 1080
 AGCTGCTTAT AA 1092

(2) INFORMATION FOR SEQUENCE ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1080

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DCR4)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 91:

ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
 GTTTGCAAAT CCGGAAACAG TGAATCAACT CAAAAATGTG GAATAGATGT TACCCTGTGT 480
 GAGGAGGCAT TCTTCAGGTT TGCTGTTTCT ACAAAGTTTA CGCCTAACTG GCTTAGTGTC 540
 TTGGTAGACA ATTTGCCTGG CACCAAAGTA AACGCAGAGA GTGTAGAGAG GATAAAACGG 600
 CAACACAGCT CACAAGAACA GACTTTCCAG CTGCTGAAGT TATGGAAACA TCAAAACAAA 660

GACCAAGATA TAGTCAAGAA GATCATCCAA GATATTGACC TCTGTGAAAA CAGCGTGCAG 720
 CGGCACATTG GACATGCTAA CCTCACCTTC GAGCAGCTTC GTAGCTTGAT GGAAAGCTTA 780
 CCGGGAAAGA AAGTGGGAGC AGAAGACATT GAAAAACAA TAAAGGCATG CAAACCCAGT 840
 GACCAGATCC TGAAGCTGCT CAGTTTGTGG CGAATAAAAA ATGGCGACCA AGACACCTTG 900
 AAGGGCCTAA TGCACGCACT AAAGCACTCA AAGACGTACC ACTTTCCCAA AACTGTCCT 960
 CAGAGTCTAA AGAAGACCAT CAGGTTTCCTT CACAGCTTCA CAATGTACAA ATTGTATCAG 1020
 AAGTTATTTT TAGAAATGAT AGGTAACCAG GTCCAATCAG TAAAAATAAG CTGCTTATAA 1080

(2) INFORMATION FOR SEQUENCE ID NO: 92:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 981

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DDD1)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 92:

ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AACCTCTCA TCAGCTGTTG 120
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATATTGAC 600
 CTCTGTGAAA ACAGCGTGCA GCGGCACATT GGACATGCTA ACCTCACCTT CGAGCAGCTT 660
 CGTAGCTTGA TGGAAAGCTT ACCGGGAAAG AAAGTGGGAG CAGAAGACAT TGAAAAACA 720
 ATAAAGGCAT GCAAACCCAG TGACCAGATC CTGAAGCTGC TCAGTTTGTG GCGAATAAAA 780
 AATGGCGACC AAGACACCTT GAAGGGCCTA ATGCACGCAC TAAAGCACTC AAAGACGTAC 840
 CACTTTCCCA AAAGTGTAC TCAGAGTCTA AAGAAGACCA TCAGGTTTCCT TCACAGCTTC 900
 ACAATGTACA AATTGTATCA GAAGTTATTT TTAGAAATGA TAGGTAACCA GGTCCAATCA 960
 GTAAAAATAA GCTGCTTATA A 981

(2) INFORMATION FOR SEQUENCE ID NO: 93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 984
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DDD2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 93:

```
ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGACG CACTAAAGCA CTCAAAGACG 840
TACCACTTTC CAAAACCTGT CACTCAGAGT CTAAAGAAGA CCATCAGGTT CCTTCACAGC 900
TTCACAATGT ACAAATTGTA TCAGAAGTTA TTTTGTAGAA TGATAGGTAA CCAGGTCCAA 960
TCAGTAAAAA TAAGCTGCTT ATAA 984
```

(2) INFORMATION FOR SEQUENCE ID NO: 94:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1200
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CL)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 94:

```
ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
```


CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAGA GCGAAATACA 420
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
 AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
 TATCAGAAGT TATTTTTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTAA 1200

(2) INFORMATION FOR SEQUENCE ID NO: 95:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1056
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CC)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 95:

ATGAACAACCT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAGA GCGAAATACA 420

GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
 AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTGA 1056

(2) INFORMATION FOR SEQUENCE ID NO: 96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 819
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CDD2)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 96:

ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
 GTGTGCGCCC CTGCCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
 AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAATGA 819

(2) INFORMATION FOR SEQUENCE ID NO: 97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 594
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CDD1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 97:

```
ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCA GA GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT ATGA 594
```

(2) INFORMATION FOR SEQUENCE ID NO: 98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 432
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CCR4)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 98:

```
ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCA GA GCGAAATACA 420
GTTTGCAAAT GA 432
```

(2) INFORMATION FOR SEQUENCE ID NO: 99:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 321

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CCR3)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 99:

```
ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG A                                     321
```

(2) INFORMATION FOR SEQUENCE ID NO: 100:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1182

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CBst)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 100:

```
ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
```

AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080
 GTCACCTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140
 TATCAGAAGT TATTTTTAGA AATGATAGGT AACCTAGTCT AG 1182

(2) INFORMATION FOR SEQUENCE ID NO: 101:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 966

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CSph)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 101:

ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
 GTTTGCAAAA GATGTCCAGA TGGGTCTTTC TCAAATGAGA CGTCATCTAA AGCACCTGT 480
 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600
 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660
 AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCTAGTCTA 960
 GACTAG

(2) INFORMATION FOR SEQUENCE ID NO: 102:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 564

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CBsp)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 102:

```
ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAG GCGAAATACA 420
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCTGT 480
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540
CACGACAACA TATGTTCCGG CTAG 564
```

(2) INFORMATION FOR SEQUENCE ID NO: 103:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 255

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-Pst)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 103:

```
ATGAACAAC T GCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
CTATACCTAG TCTAG 255
```

(2) INFORMATION FOR SEQUENCE ID NO: 104:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1317

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : human OCIF genomic DNA-1

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 104:

```
CTGGAGACAT ATAACTTGAA CACTTGGCCC TGATGGGGAA GCAGCTCTGC AGGGACTTTT    60
TCAGCCATCT GTAAACAATT TCAGTGGCAA CCCGCGAACT GTAATCCATG AATGGGACCA    120
CACTTTACAA GTCATCAAGT CTAAC TTCTA GACCAGGGAA TTAATGGGGG AGACAGCGAA    180
CCCTAGAGCA AAGTGCCAAA CTTCTGTCGA TAGCTTGAGG CTAGTGGAAA GACCTCGAGG    240
AGGCTACTCC AGAAGTTCAG CGCGTAGGAA GCTCCGATAC CAATAGCCCT TTGATGATGG    300
TGGGGTTGGT GAAGGGAACA GTGCTCCGCA AGGTTATCCC TGCCCCAGGC AGTCCAATTT    360
TCACTCTGCA GATTCTCTCT GGCTCTAACT ACCCCAGATA ACAAGGAGTG AATGCAGAAT    420
AGCACGGGCT TTAGGGCCAA TCAGACATTA GTTAGAAAAA TTCCTACTAC ATGGTTTATG    480
TAAACTTGAA GATGAATGAT TGCGAAC TCC CCGAAAAGGG CTCAGACAAT GCCATGCATA    540
AAGAGGGGCC CTGTAATTTG AGGTTTCAGA ACCCGAAGTG AAGGGGTCAG GCAGCCGGGT    600
ACGGCGGAAA CTCACAGCTT TCGCCCAGCG AGAGGACAAA GGTCTGGGAC AACTCCAAC    660
TGCGTCCGGA TCTTGGCTGG ATCGGACTCT CAGGGTGGAG GAGACACAAG CACAGCAGCT    720
GCCCAGCGTG TGCCCAGCCC TCCCACCGCT GGTCCCGGCT GCCAGGAGGC TGGCCGCTGG    780
CGGGAAGGGG CCGGGAACC TCAGAGCCCC GCGGAGACAG CAGCCGCCTT GTTCCTCAGC    840
CCGGTGGCTT TTTTTTCCC TGCTCTCCCA GGGGACAGAC ACCACCGCCC CACCCCTCAC    900
GCCCCACCTC CCTGGGGGAT CCTTTCGCC CCAGCCCTGA AAGCGTTAAT CCTGGAGCTT    960
TCTGCACACC CCCCAGCCGC TCCCGCCCAA GCTTCCTAAA AAAGAAAGGT GCAAAGTTTG   1020
GTCCAGGATA GAAAAATGAC TGATCAAAGG CAGGCGATAC TTCCTGTTGC CGGGACGCTA   1080
TATATAACGT GATGAGCGCA CGGGCTGCGG AGACGCACCG GAGCGCTCGC CCAGCCGCCG   1140
CCTCCAAGCC CCTGAGGTTT CCGGGGACCA CA ATG AAC AAG TTG CTG TGC TGC   1193
```

Met Asn Lys Leu Leu Cys Cys

-20

-15

```
GCG CTC GTG GTAAGTCCCT GGGCCAGCCG ACGGGTGCCC GGCGCCTGGG    1242
Ala Leu Val
```

```
GAGGCTGCTG CCACCTGGTC TCCCAACCTC CCAGCGGACC GGCGGGGAAA AAGGCTCCAC    1302
```

(2) INFORMATION FOR SEQUENCE ID NO: 105:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH :

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : human OCIF genomic DNA-2

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 105:

GCTTACTTTG TGCCAAATCT CATTAGGCTT AAGGTAATAC AGGACTTTGA GTCAAATGAT	@60
ACTGTTGCAC ATAAGAACAA ACCTATTTTC ATGCTAAGAT GATGCCACTG TGTCCTTTC	120
TCCTTCTAG TTT CTG GAC ATC TCC ATT AAG TGG ACC ACC CAG GAA ACG TTT	171
Phe Leu Asp Ile Ser Ile Lys Trp Thr Thr Gln Glu Thr Phe	
-10 -5 -1 1	
CCT CCA AAG TAC CTT CAT TAT GAC GAA GAA ACC TCT CAT CAG CTG TTG	219
Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His Gln Leu Leu	
5 10 15	
TGT GAC AAA TGT CCT CCT GGT ACC TAC CTA AAA CAA CAC TGT ACA GCA	267
Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala	
20 25 30 35	
AAG TGG AAG ACC GTG TGC GCC CCT TGC CCT GAC CAC TAC TAC ACA GAC	315
Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp	
40 45 50	
AGC TGG CAC ACC AGT GAC GAG TGT CTA TAC TGC AGC CCC GTG TGC AAG	363
Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys	
55 60 65	
GAG CTG CAG TAC GTC AAG CAG GAG TGC AAT CGC ACC CAC AAC CGC GTG	411
Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val	
70 75 80	

TGC GAA TGC AAG GAA GGG CGC TAC CTT GAG ATA GAG TTC TGC TTG AAA	459
Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys	
85 90 95	
CAT AGG AGC TGC CCT CCT GGA TTT GGA GTG GTG CAA GCT G GTACGTGTCA	509
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala	
100 105 110	
ATGTGCAGCA AAATTAATTA GGATCATGCA AAGTCAGATA GTTGTGACAG TTTAGGAGAA	569
CACTTTTGTG CTGATGACAT TATAGGATAG CAAATTGCAA AGGTAATGAA ACCTGCCAGG	629
TAGGTACTAT GTGTCTGGAG TGCTTCCAAA GGACCATTGC TCAGAGGAAT ACTTTGCCAC	689
TACAGGGCAA TTTAATGACA AATCTCAAAT GCAGCAAATT ATTCTCTCAT GAGATGCATG	749
ATGGTTTTTT TTTTTTTTTT TAAAGAAACA AACTCAAGTT GCACTATTGA TAGTTGATCT	809
ATACCTCTAT ATTTCACTTC AGCATGGACA CCTTCAAAC TGCAGCACTTT TTGACAAACA	869
TCAGAAATGT TAATTTATAC CAAGAGAGTA ATTATGCTCA TATTAATGAG ACTCTGGAGT	929
GCTAACAATA AGCAGTTATA ATTAATTATG TAAAAAATGA GAATGGTGAG GGAATTGCA	989
TTTCATTATT AAAAACAAGG CTAGTTCTTC CTTTAGCATG GGAGCTGAGT GTTTGGGAGG	1049
GTAAGGACTA TAGCAGAATC TCTTCAATGA GCTTATTCTT TATCTTAGAC AAAACAGATT	1109
GTCAAGCCAA GAGCAAGCAC TTGCCTATAA ACCAAGTGCT TTCTCTTTTG CATTTTGAAC	1169
AGCATTGGTC AGGGCTCATG TGTATTGAAT CTTTAAACC AGTAACCCAC GTTTTTTTTC	1229
TGCCACATTT GCGAAGCTTC AGTGCAGCCT ATAACCTTTC ATAGCTTGAG AAAATTAAGA	1289
GTATCCACTT ACTTAGATGG AAGAAGTAAT CAGTATAGAT TCTGATGACT CAGTTTGAAG	1349
CAGTGTTTCT CAACTGAAGC CCTGCTGATA TTTTAAGAAA TATCTGGATT CCTAGGCTGG	1409
ACTCCTTTTT GTGGGCAGCT GTCCTGCGCA TTGTAGAATT TTGGCAGCAC CCCTGGACTC	1469
TAGCCACTAG ATACCAATAG CAGTCCTTCC CCCATGTGAC AGCCAAAAAT GTCTTCAGAC	1529
ACTGTCAAAT GTCGCCAGGT GGCAAAATCA CTCCTGGTTG AGAACAGGGT CATCAATGCT	1589
AAGTATCTGT AACTATTTTA ACTCTCAAAA CTTGTGATAT ACAAAGTCTA AATTATTAGA	1649
CGACCAATAC TTTAGGTTTA AAGGCATACA AATGAAACAT TCAAAAATCA AAATCTATTC	1709
TGTTTCTCAA ATAGTGAATC TTATAAAATT AATCACAGAA GATGCAAATT GCATCAGAGT	1769
CCCTTAAAT TCCTCTTCGT ATGAGTATTT GAGGGAGGAA TTGGTGATAG TTCCTACTTT	1829
CTATTGGATG GTACTTTGAG ACTCAAAAGC TAAGCTAAGT TGTGTGTGTG TCAGGGTGCG	1889
GGGTGTGGAA TCCCATCAGA TAAAAGCAAA TCCATGTAAT TCATTCAGTA AGTTGTATAT	1949
GTAGAAAAAT GAAAAGTGGG CTATGCAGCT TGGAAACTAG AGAATTTTGA AAAATAATGG	2009
AAATCACAAG GATCTTTCTT AAATAAGTAA GAAAATCTGT TTGTAGAATG AAGCAAGCAG	2069
GCAGCCAGAA GACTCAGAAC AAAAGTACAC ATTTTACTCT GTGTACACTG GCAGCACAGT	2129
GGGATTTATT TACCTCTCCC TCCCTAAAAA CCCACACAGC GGTTCTCTT GGGAAATAAG	2189

AGGTTTCCAG	CCCAAAGAGA	AGGAAAGACT	ATGTGGTGTT	ACTCTAAAAA	GTATTTAATA	2249
ACCGTTTTGT	TGTTGCTGTT	GCTGTTTTGA	AATCAGATTG	TCTCCTCTCC	ATATTTTATT	2309
TACTTCATTC	TGTTAATTCC	TGTGGAATTA	CTTAGAGCAA	GCATGGTGAA	TTCTCAACTG	2369
TAAAGCCAAA	TTTCTCCATC	ATTATAATTT	CACATTTTGC	CTGGCAGGTT	ATAATTTTTA	2429
TATTTCCACT	GATAGTAATA	AGGTAAAATC	ATTACTTAGA	TGGATAGATC	TTTTTCATAA	2489
AAAGTACCAT	CAGTTATAGA	GGGAAGTCAT	GTTTCATGTT	AGGAAGGTCA	TTAGATAAAG	2549
CTTCTGAATA	TATTATGAAA	CATTAGTTCT	GTCATTCTTA	GATTCTTTTT	GTAAATAAAC	2609
TTTAAAAGCT	AACTTACCTA	AAAGAAATAT	CTGACACATA	TGAACTTCTC	ATTAGGATGC	2669
AGGAGAAGAC	CCAAGCCACA	GATATGTATC	TGAAGAATGA	ACAAGATTCT	TAGGCCCGGC	2729
ACGGTGGCTC	ACATCTGTAA	TCTCAAGAGT	TTGAGAGGTC	AAGGCGGGCA	GATCACCTGA	2789
GGTCAGGAGT	TCAAGACCAG	CCTGGCCAAC	ATGATGAAAC	CCTGCCTCTA	CTAAAAATAC	2849
AAAAATTAGC	AGGGCATGGT	GGTGCATGCC	TGCAACCCTA	GCTACTCAGG	AGGCTGAGAC	2909
AGGAGAATCT	CTTGAACCCT	CGAGGCGGAG	GTTGTGGTGA	GCTGAGATCC	CTCTACTGCA	2969
CTCCAGCCTG	GGTGACAGAG	ATGAGACTCC	GTCCCTGCCG	CCGCCCCCGC	CTTCCCCCCC	3029
AAAAAGATTC	TTCTTCATGC	AGAACATACG	GCAGTCAACA	AAGGGAGACC	TGGGTCCAGG	3089
TGTCCAAGTC	ACTTATTTTCG	AGTAAATTAG	CAATGAAAGA	ATGCCATGGA	ATCCCTGCCC	3149
AAATACCTCT	GCTTATGATA	TTGTAGAATT	TGATATAGAG	TTGTATCCCA	TTTAAGGAGT	3209
AGGATGTAGT	AGGAAAGTAC	TAAAAACAAA	CACACAAACA	GAAAACCCTC	TTTGCTTTGT	3269
AAGGTGGTTC	CTAAGATAAT	GTCAGTGCAA	TGCTGGAAAT	AATATTTAAT	ATGTGAAGGT	3329
TTTAGGCTGT	GTTTTCCCCT	CCTGTTCTTT	TTTTCTGCCA	GCCCTTTGTC	ATTTTGCAG	3389
GTCAATGAAT	CATGTAGAAA	GAGACAGGAG	ATGAAACTAG	AACCAGTCCA	TTTTGCCCTT	3449
TTTTTTATTT	TCTGGTTTTG	GTAAGAGATA	CAATGAGGTA	GGAGGTTGAG	ATTTATAAAT	3509
GAAGTTTAAT	AAGTTTCTGT	AGCTTTGATT	TTTCTCTTTC	ATATTTGTTA	TCTTGCATAA	3569
GCCAGAATTG	GCCTGTAAAA	TCTACATATG	GATATTGAAG	TCTAAATCTG	TTCAACTAGC	3629
TTACACTAGA	TGGAGATATT	TTCATATTCA	GATACACTGG	AATGTATGAT	CTAGCCATGC	3689
GTAATATAGT	CAAGTGTTTG	AAGGTATTTA	TTTTTAATAG	CGTCTTTAGT	TGTGGACTGG	3749
TTCAAGTTTT	TCTGCCAATG	ATTTCTTCAA	ATTTATCAAA	TATTTTTCCT	TCATGAAGTA	3809
AAATGCCCTT	GCAGTCACCC	TTCCTGAAGT	TTGAACGACT	CTGCTGTTTT	AAACAGTTTA	3869
AGCAAATGGT	ATATCATCTT	CCGTTTACTA	TGTAGCTTAA	CTGCAGGCTT	ACGCTTTTGA	3929
GTCAGCGGCC	AACTTTATTG	CCACCTTCAA	AAGTTTATTA	TAATGTTGTA	AATTTTACT	3989
TCTCAAGGTT	AGCATACTTA	GGAGTTGCTT	CACAATTAGG	ATTCAGGAAA	GAAAGAACTT	4049
CAGTAGGAAC	TGATTGGAAT	TTAATGATGC	AGCATTCAAT	GGGTACTAAT	TTCAAAGAAT	4109
GATATTACAG	CAGACACACA	GCAGTTATCT	TGATTTTCTA	GGAATAATTG	TATGAAGAAT	4169
ATGGCTGACA	ACACGGCCTT	ACTGCCACTC	AGCGGAGGCT	GGACTAATGA	ACACCCTACC	4229
CTTCTTTCCT	TTCCTCTCAC	ATTTTCATGAG	CGTTTTGTAG	GTAACGAGAA	AATTGACTTG	4289
CATTTGCATT	ACAAGGAGGA	GAAACTGGCA	AAGGGGATGA	TGGTGGAAGT	TTTGTTCTGT	4349

CTAATGAAGT	GAAAAATGAA	AATGCTAGAG	TTTTGTGCAA	CATAATAGTA	GCAGTAAAAA	4409
CCAAGTGAAA	AGTCTTTCCA	AAACTGTGTT	AAGAGGGCAT	CTGCTGGGAA	ACGATTTGAG	4469
GAGAAGGTAC	TAAATTGCTT	GGTATTTTCC	GTAG GA ACC	CCA GAG CGA	AAT ACA	4523

Gly Thr Pro Glu Arg Asn Thr

GTT TGC AAA AGA TGT CCA GAT GGG TTC TTC TCA AAT GAG ACG TCA TCT 4571
Val Cys Lys Arg Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser
120 125 130 135

AAA GCA CCC TGT AGA AAA CAC ACA AAT TGC AGT GTC TTT GGT CTC CTG 4619
Lys Ala Pro Cys Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu
140 145 150

CTA ACT CAG AAA GGA AAT GCA ACA CAC GAC AAC ATA TGT TCC GGA AAC 4667
Leu Thr Gln Lys Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn
155 160 165

AGT GAA TCA ACT CAA AAA TGT GGA ATA G GTAATTACAT TCCAAAATAC 4715
Ser Glu Ser Thr Gln Lys Cys Gly Ile
170 175

GTCTTTGTAC	GATTTTGTAG	TATCATCTCT	CTCTCTGAGT	TGAACACAAG	GCCTCCAGCC	4775
ACATTCTTGG	TCAAACCTTAC	ATTTTCCCTT	TCTTGAATCT	TAACCAGCTA	AGGCTACTCT	4835
CGATGCATTA	CTGCTAAAGC	TACCACTCAG	AATCTCTCAA	AAACTCATCT	TCTCACAGAT	4895
AACACCTCAA	AGCTTGATTT	TCTCTCCTTT	CACACTGAAA	TCAAATCTTG	CCCATAGGCA	4955
AAGGGCAGTG	TCAAGTTTGC	CACTGAGATG	AAATTAGGAG	AGTCCAAACT	GTAGAATTCA	5015
CGTTGTGTGT	TATTACTTTC	ACGAATGTCT	GTATTATTAA	CTAAAGTATA	TATTGGCAAC	5075
TAAGAAGCAA	AGTGATATAA	ACATGATGAC	AAATTAGGCC	AGGCATGGTG	GCTTACTCCT	5135
ATAATCCCAA	CATTTTGGGG	GGCCAAGGTA	GGCAGATCAC	TTGAGGTCAG	GATTTCAAGA	5195
CCAGCCTGAC	CAACATGGTG	AAACCTTGTC	TCTACTAAAA	ATACAAAAAT	TAGCTGGGCA	5255
TGGTAGCAGG	CACTTCTAGT	ACCAGCTACT	CAGGGCTGAG	GCAGGAGAAT	CGCTTGAACC	5315
CAGGAGATGG	AGGTTGCAGT	GAGCTGAGAT	TGTACCACTG	CACTCCAGTC	TGGGCAACAG	5375
AGCAAGATTT	CATCACACAC	ACACACACAC	ACACACACAC	ACACATTAGA	AATGTGTACT	5435
TGGCTTTGTT	ACCTATGGTA	TTAGTGCATC	TATTGCATGG	AACTTCCAAG	CTACTCTGGT	5495
TGTGTTAAGC	TCTTCATTGG	GTACAGGTCA	CTAGTATTAA	GTTCAGGTTA	TTCGGATGCA	5555

TTCCACGGTA	GTGATGACAA	TTCATCAGGC	TAGTGTGTGT	GTTACACCTTG	TCACTCCCAC	5615
CACTAGACTA	ATCTCAGACC	TTCACTCAA	GACACATTAC	ACTAAAGATG	ATTTGCTTTT	5675
TTGTGTTTAA	TCAAGCAATG	GTATAAACCA	GCTTGACTCT	CCCCAAACAG	TTTTTCGTAC	5735
TACAAAGAAG	TTTATGAAGC	AGAGAAATGT	GAATTGATAT	ATATATGAGA	TTCTAACCCA	5795
GTTCCAGCAT	TGTTTCATTG	TGTAATTGAA	ATCATAGACA	AGCCATTTTA	GCCTTTGCTT	5855
TCTTATCTAA	AAAAAAAAAA	AAAAAATGA	AGGAAGGGGT	ATTAAGAGGA	GTGATCAAAT	5915
TTTAACATTC	TCTTTAATTA	ATTCATTTTT	AATTTTACTT	TTTTTCATTT	ATTGTGCACT	5975
TACTATGTGG	TACTGTGCTA	TAGAGGCTTT	AACATTTATA	AAAACACTGT	GAAAGTTGCT	6035
TCAGATGAAT	ATAGGTAGTA	GAACGGCAGA	ACTAGTATTC	AAAGCCAGGT	CTGATGAATC	6095
CAAAAACAAA	CACCCATTAC	TCCCATTTTC	TGGGACATAC	TACTCTACC	CAGATGCTCT	6155
GGGCTTTGTA	ATGCCTATGT	AAATAACATA	GTTTTATGTT	TGGTTATTTT	CCTATGTAAT	6215
GTCTACTTAT	ATATCTGTAT	CTATCTCTTG	CTTTGTTTCC	AAAGGTAAAC	TATGTGTCTA	6275
AATGTGGGCA	AAAAATAACA	CACTATTCCA	AATTACTGTT	CAAATTCCTT	TAAGTCAGTG	6335
ATAATTATTT	GTTTTGACAT	TAATCATGAA	GTTCCCTGTG	GGTACTAGGT	AAACCTTTAA	6395
TAGAATGTTA	ATGTTTGTAT	TCATTATAAG	AATTTTTGGC	TGTTACTTAT	TTACAACAAT	6455
ATTTCACTCT	AATTAGACAT	TTACTAAACT	TTCTCTTGAA	AACAATGCCC	AAAAAAGAAC	6515
ATTAGAAGAC	ACGTAAGCTC	AGTTGGTCTC	TGCCACTAAG	ACCAGCCAAC	AGAAGCTTGA	6575
TTTTATTCAA	ACTTTGCATT	TTAGCATATT	TTATCTTGGA	AAATTCAATT	GTGTTGGTTT	6635
TTTGTTTTTG	TTTGTATTGA	ATAGACTCTC	AGAAATCCAA	TTGTTGAGTA	AATCTTCTGG	6695
GTTTTCTAAC	CTTTCTTTAG	AT GTT ACC	CTG TGT GAG	GAG GCA TTC	TTC AGG	6747

Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
 180 185

TTT GCT GTT CCT ACA AAG TTT ACG CCT AAC TGG CTT AGT GTC TTG GTA	6795
Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val	
190 195 200	

GAC AAT TTG CCT GGC ACC AAA GTA AAC GCA GAG AGT GTA GAG AGG ATA	6843
Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile	
205 210 215	

AAA CGG CAA CAC AGC TCA CAA GAA CAG ACT TTC CAG CTG CTG AAG TTA	6891
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu	
220 225 230 235	

TGG AAA CAT CAA AAC AAA GAC CAA GAT ATA GTC AAG AAG ATC ATC CAA G	6940
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Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln
240 245 250

GTAATTACAT	TCCAAAATAC	GTCTTTGTAC	GATTTTGTAG	TATCATCTCT	CTCTCTGAGT	7000
TGAACACAAG	GCCTCCAGCC	ACATTCTTGG	TCAAACCTTAC	ATTTTCCCTT	TCTTGAATCT	7060
TAACCAGCTA	AGGCTACTCT	CGATGCATTA	CTGCTAAAGC	TACCACTCAG	AATCTCTCAA	7120
AAACTCATCT	TCTCACAGAT	AACACCTCAA	AGCTTGATTT	TCTCTCCTTT	CACACTGAAA	7180
TCAAATCTTG	CCCATAGGCA	AAGGGCAGTG	TCAAGTTTGC	CACTGAGATG	AAATTAGGAG	7240
AGTCCAAACT	GTAGAATTCA	CGTTGTGTGT	TATTACTTTC	ACGAATGTCT	GTATTATTAA	7300
CTAAAGTATA	TATTGGCAAC	TAAGAAGCAA	AGTGATATAA	ACATGATGAC	AAATTAGGCC	7360
AGGCATGGTG	GCTTACTCCT	ATAATCCCAA	CATTTTGGGG	GGCCAAGGTA	GGCAGATCAC	7420
TTGAGGTCAG	GATTTCAAGA	CCAGCCTGAC	CAACATGGTG	AAACCTTGTC	TCTACTAAAA	7480
ATACAAAAAT	TAGCTGGGCA	TGGTAGCAGG	CACTTCTAGT	ACCAGCTACT	CAGGGCTGAG	7540
GCAGGAGAAT	CGCTTGAACC	CAGGAGATGG	AGGTTGCACT	GAGCTGAGAT	TGTACCACTG	7600
CACTCCAGTC	TGGGCAACAG	AGCAAGATTT	CATCACACAC	ACACACACAC	ACACACACAC	7660
ACACATTAGA	AATGTGTACT	TGGCTTTGTT	ACCTATGGTA	TTAGTGCATC	TATTGCATGG	7720
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GTTCAGGTTA	TTCGGATGCA	TTCCACGGTA	GTGATGACAA	TTCATCAGGC	TAGTGTGTGT	7840
GTTCACCTTG	TCACTCCCAC	CACTAGACTA	ATCTCAGACC	TTCACTCAAA	GACACATTAC	7900
ACTAAAGATG	ATTTGCTTTT	TTGTGTTTAA	TCAAGCAATG	GTATAAACCA	GCTTGACTCT	7960
CCCCAAACAG	TTTTTCGTAC	TACAAAGAAG	TTTATGAAGC	AGAGAAATGT	GAATTGATAT	8020
ATATATGAGA	TTCTAACCCA	GTTCCAGCAT	TGTTTCATTG	TGTAATTGAA	ATCATAGACA	8080
AGCCATTTTA	GCCTTTGCTT	TCTTATCTAA	AAAAAAAAAA	AAAAAAATGA	AGGAAGGGGT	8140
ATTAAAAGGA	GTGATCAAAT	TTTAACATTC	TCTTTAATTA	ATTCATTTTT	AATTTTACTT	8200
TTTTTCATTT	ATTGTGCACT	TACTATGTGG	TACTGTGCTA	TAGAGGCTTT	AACATTTATA	8260
AAAACACTGT	GAAAGTTGCT	TCAGATGAAT	ATAGGTAGTA	GAACGGCAGA	ACTAGTATTC	8320
AAAGCCAGGT	CTGATGAATC	CAAAAACAAA	CACCCATTAC	TCCCATTTTC	TGGGACATAC	8380
TTACTCTACC	CAGATGCTCT	GGGCTTTGTA	ATGCCTATGT	AAATAACATA	GTTTTATGTT	8440
TGGTTATTTT	CCTATGTAAT	GTCTACTTAT	ATATCTGTAT	CTATCTCTTG	CTTTGTTTCC	8500
AAAGGTAAAC	TATGTGTCTA	AATGTGGGCA	AAAAATAACA	CACTATTCCA	AATTACTGTT	8560
CAAATTCCTT	TAAGTCAGTG	ATAATTATTT	GTTTTGACAT	TAATCATGAA	GTTCCCTGTG	8620
GGTACTAGGT	AAACCTTTAA	TAGAATGTTA	ATGTTTGTAT	TCATTATAAG	AATTTTGGC	8680
TGTTACTTAT	TTACAACAAT	ATTTCACTCT	AATTAGACAT	TTACTAAACT	TTCTCTTGAA	8740
AACAATGCCC	AAAAAGAAC	ATTAGAAGAC	ACGTAAGCTC	AGTTGGTCTC	TGCCACTAAG	8800
ACCAGCCAAC	AGAAGCTTGA	TTTTATTCAA	ACTTTGCATT	TTAGCATATT	TTATCTTGGA	8860
AAATTCAATT	GTGTTGGTTT	TTTGTTTTTG	TTTGTATTGA	ATAGACTCTC	AGAAATCCAA	8920

TTGTTGAGTA AATCTTCTGG GTTTTCTAAC CTTTCTTTAG AT ATT GAC CTC TGT	8974
Asp Ile Asp Leu Cys	
255	
GAA AAC AGC GTG CAG CGG CAC ATT GGA CAT GCT AAC CTC ACC TTC GAG	9022
Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu	
260 265 270	
CAG CTT CGT AGC TTG ATG GAA AGC TTA CCG GGA AAG AAA GTG GGA GCA	9070
Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala	
275 280 285	
GAA GAC ATT GAA AAA ACA ATA AAG GCA TGC AAA CCC AGT GAC CAG ATC	9118
Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile	
290 295 300	
CTG AAG CTG CTC AGT TTG TGG CGA ATA AAA AAT GGC GAC CAA GAC ACC	9166
Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr	
305 310 315 320	
TTG AAG GGC CTA ATG CAC GCA CTA AAG CAC TCA AAG ACG TAC CAC TTT	9214
Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe	
325 330 335	
CCC AAA ACT GTC ACT CAG AGT CTA AAG AAG ACC ATC AGG TTC CTT CAC	9262
Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His	
340 345 350	
AGC TTC ACA ATG TAC AAA TTG TAT CAG AAG TTA TTT TTA GAA ATG ATA	9310
Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile	
355 360 365	
GGT AAC CAG GTC CAA TCA GTA AAA ATA AGC TGC TTA TAACTGGAAA	9356
Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu	
370 375 380	

TGGCCATTGA	GCTGTTTCCT	CACAATTGGC	GAGATCCCAT	GGATGAGTAA	ACTGTTTCTC	9416
AGGCACTTGA	GGCTTTCAGT	GATATCTTTC	TCATTACCAG	TGACTAATTT	TGCCACAGGG	9476
TACTAAAAGA	AACTATGATG	TGGAGAAAGG	ACTAACATCT	CCTCCAATAA	ACCCCAAATG	9536
GTTAATCCAA	CTGTCAGATC	TGGATCGTTA	TCTACTGACT	ATATTTTCCC	TTATTACTGC	9596
TTGCAGTAAT	TCAACTGGAA	ATTAAAAAAA	AAAAACTAGA	CTCCACTGGG	CCTTACTAAA	9656
TATGGGAATG	TCTAACTTAA	ATAGCTTTGG	GATTCCAGCT	ATGCTAGAGG	CTTTTATTAG	9716
AAAGCCATAT	TTTTTCTGT	AAAAGTTACT	AATATATCTG	TAACACTATT	ACAGTATTGC	9776
TATTTATATT	CATTCAGATA	TAAGATTTGG	ACATATTATC	ATCCTATAAA	GAAACGGTAT	9836
GACTTAATTT	TAGAAAGAAA	ATTATATTCT	GTTTATTATG	ACAAATGAAA	GAGAAAATAT	9896
ATATTTTAA	TGGAAAGTTT	GTAGCATTTT	TCTAATAGGT	ACTGCCATAT	TTTTCTGTGT	9956
GGAGTATTTT	TATAATTTTA	TCTGTATAAG	CTGTAATATC	ATTTTATAGA	AAATGCATTA	10016
TTTAGTCAAT	TGTTTAATGT	TGGAAAACAT	ATGAAATATA	AATTATCTGA	ATATTAGATG	10076
CTCTGAGAAA	TTGAATGTAC	CTTATTTAAA	AGATTTTATG	GTTTTATAAC	TATATAAATG	10136
ACATTATTAA	AGTTTTCAAA	TTATTTTTTA	TTGCTTTCTC	TGTTGCTTTT	ATTT	10190